CLINICAL PROSPECTIVE CROSS SECTIONAL STUDY TO EXPLORE THE RELATIONSHIP BETWEEN HYPOTHYROIDISM AND HORMONE THERAPY WITH RESPECT TO CHRONIC INFLAMMATION

DISSERTATION

SUBMITTED FOR

M.D. PHARMACOLOGY

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

DEPARTMENT OF PHARMACOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

PEELAMEDU, COIMBATORE – 641004

TAMILNADU, INDIA

APRIL - 2017

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

COIMBATORE

CERTIFICATE

This is to certify that this dissertation entitled **"Clinical Prospective Cross Sectional Study to Explore the Relationship Between Hypothyroidism and Hormone Therapy with Respect to Chronic Inflammation"**, is a work done by **Dr.R.Muthamizhveena,** Postgraduate, under the guidance of **Dr.K.Bhuvaneswari, M.D**., Professor and Head, Department of Pharmacology, PSG IMS&R.

Dr. K.Bhuvaneswari M.D.
Guide, Professor and Head,
Department of Pharmacology,
PSG IMS&R.

Dr.S.Ramalingam M.D.
Dean
PSG IMS&R.

DECLARATION

I solemnly declare that the dissertation titled **"Clinical Prospective Cross Sectional Study To Explore The Relationship Between Hypothyroidism And Hormone Therapy With Respect To Chronic Inflammation"** was done by me under the guidance and supervision of **Dr.K.Bhuvaneswari,M.D.**

This dissertation is submitted to the Tamilnadu Dr.MGR Medical University towards the partial fulfilment of the requirement for the award of M.D. Degree in Pharmacology.

Place: **Dr.R.Muthamizhveena**

Date:

ACKNOWLEDGEMENT

I express my gratitude and sincere thanks to Dr.K.Bhuvaneswari, Professor and Head, Department of Pharmacology, PSGIMS&R for being my guide. It was her valuable suggestions and constant encouragement in every step that has helped me to complete my research work successfully. I could not have imagined having a better advisor and mentor in my carrier. I can rightfully say that without her, none of this work would have been possible.

I express my sincere thanks to Dr.S.Ramalingam, (Dean & Professor of Pharmacology, PSGIMSR), and Dr.G.Jeyachandran (HOD, department of Biochemistry) for the necessary permissions granted and for the excellent amenities offered to carry out my study.

I am obliged to thank Dr.K.Jeyachandran, Dr. Senthil kumar and Dr.Suresh prabu, PSG Hospitals for their constant support in recruiting study participants.

My sincere thanks to Professor Dr.S.Bhuvaneshwari M.D., Professor Dr.T.K.Ponnusamy M.D., Associate professor Dr.C.Deena Sangeetha M.D, Assistant professors Dr.N.Ramanujam M.D, Dr.S.Shanmugapriya M.D, and Senior resident Dr.G.Amudha for their advice and encouragement.

I wish to express my whole hearted thanks to my seniors especially Dr.S.Breetha and Dr.E.Amudhan Arvind for giving me emotional support and having helped me in all the time of my research work.

I also thank my fellow postgraduate Dr.Jeyalakshmi and my junior postgraduates Dr.Yamuna devi, Dr.Bindu, Dr.Marymala and Dr.Karthika for their support to complete the project on time.

I am indebted to all the patients of my study for their kind participation and to all the technical staff members of Pharmacology and Biochemistry departments for having spared their time and effort towards my research work.

Last but not the least; I would like to thank my parents, my sister, my in-laws and my husband for their love and patience when I was held up with my work. All the support they have provided me over the years was the greatest gift anyone has ever given me.

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Institutional Human Ethics Committee

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Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

To
Dr R Muthamizh Veena
Postgraduate
Department of Pharmacology
PSG IMS & R
Coimbatore

Ref: Project No. 14/430

Date: December 18, 2014

Dear Dr Muthamizh Veena,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 11.12.2014 to conduct the research study entitled "*Clinical prospective cross sectional study to explore the relationship between hypothyroidism and hormone therapy with respect to chronic inflammation*" during the IHEC meeting held on 12.12.2014.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol
3. Informed consent forms
4. Proforma
5. Permission letter from concerned Head of the department
6. Current CVs of Principal investigator, Co-investigators
7. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 12.12.2014 at IHEC Secretariat, PSG IMS & R between 10.00 am and 11.00 am:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Dr. P. Sathyan (Chairperson, IHEC)	DO, DNB	Clinician (Ophthalmology)	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr. S.Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

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Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
   e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC and only then can they be implemented
   f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review
7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Thanking You,

Yours Sincerely,

15-12-14

Dr S Bhuvaneshwari
Member-Secretary
Institutional Human Ethics Committee

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PSG IMS&R
COIMBATORE-641004.
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December 8, 2015

To
Dr R Muthamizhveena
Postgraduate
Department of Pharmacology
Guide: Dr K Bhuvaneswari
PSG IMS & R
Coimbatore

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 4th December, 2015 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your request to renew the approval for the study entitled:

"Clinical prospective cross sectional study to explore the relationship between hypothyroidism and hormone therapy with respect to chronic inflammation"

The following documents were received for review:

1. Request for renewal dated 03.12.2015
2. Status report

After due consideration, the Committee has decided to renew the approval for the study.

The members who attended the meeting held on at which your proposal was discussed, are listed below:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Mr. R. Nandakumar	BA., BL	Legal Expert, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist Member – Secretary	Female	Yes	Yes
Dr S Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
Dr Sudha Ramalingam	MD	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The approval is valid for one year (18.12.2015 to 17.12.2016).

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

SECRETARY
PSG IMS&R
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INSTITUTIONAL HUMAN ETHICS COMMITTEE

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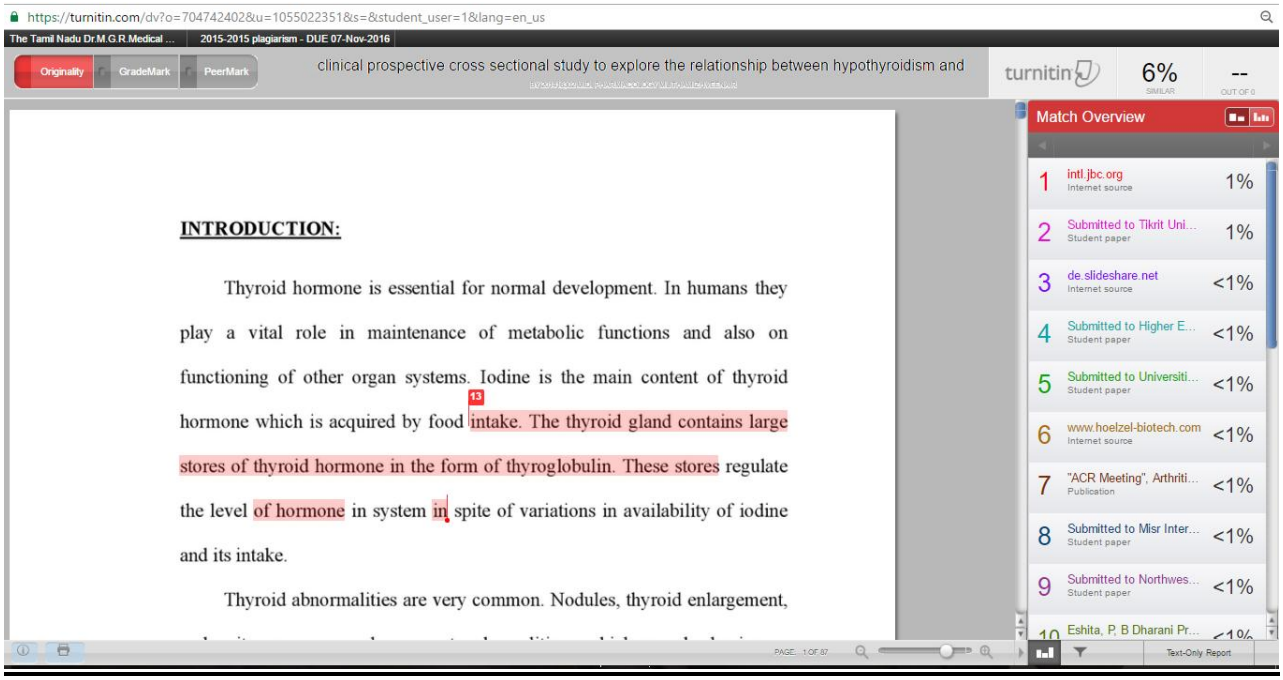
INTRODUCTION

Thyroid hormone is essential for normal development. It hormone they play a vital role in maintenance of metabolic functions and also on functioning of other organ systems. Iodine is the main content of thyroid hormone which is acquired by food intake. The thyroid gland contains large stores of thyroid hormone in the form of tyroglobulin. These stores regulate the level of hormone in system in spite of variations in availability of iodine and its intake.

Thyroid abnormalities are very common. Nodules, thyroid enlargement, and goiter are commonly encountered conditions which may be benign or malignant. Mostly in these conditions hormone levels in circulation will be in euthyroid range. Thyroid hormone changes, beta thyroid and hypothyroidism are mostly associated with predominant clinical manifestations. Mild stage of disease will be presenting often with subtle clinical situation, which is confirmed by required laboratory tests. Here been screening has been done now in developed countries for congenital hypothyroidism, so that early institution of appropriate hormone replacement therapy is given which has reduced mental retardation and serious due hormonal deficiency.

Hypothyroidism is a condition characterized by abnormally low thyroid hormone production. They are the common endocrine disorder with prevalence of 4-10% in adults. In Indian adults the prevalence hypothyroidism was less than 10%. A study done in Cochin revealed prevalence of hypothyroidism as 3.9%. Also there was high prevalence of subclinical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Thyroid abnormalities are very common. Nodules, thyroid enlargement,
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Introduction

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Thyroid abnormalities are very common. Nodules, thyroid enlargement, and goitre are commonly encountered conditions which may be benign or malignant. Mostly in these conditions hormone levels in circulation will be in reference range. Thyroid hormone changes, both hyper and hypothyroidism are mostly associated with predominant clinical manifestations. Mild stage of disease will be presenting often with subtle clinical situation, which is confirmed by atypical laboratory tests. New born screening has been done now in developed countries for congenital hypothyroidism, so that early institution of appropriate hormone replacement therapy is given which has reduced mental retardation and cretinism due to hormonal deficiency.

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hypothyroidism with 9.4%.Women: Men prevalence in that study was 11.4% and 6.2% respectively[1].

Thyroid hormones are necessary for normal development of organs. Overactive thyroid ending up in increased release of T3 and T4 in bloodstream ends up in thyrotoxicosis. Hypothyroidism is the reverse condition. Primary hypothyroidism is due to problem in thyroid gland itself while secondary hypothyroidism is due to inadequate functioning of "hypothalamic-pituitary-thyroid-axis". Iodine deficiency is the commonest global reason of hypothyroidism.

Hypothyroidism is a common condition and harmful effects are caused on cardiovascular system both by overt and subclinical hypothyroidism[2]. This is caused by several different mechanisms which cause increased risks of atherosclerosis and coronary heart disease[3, 4].

A number of inflammatory biomarkers in circulation are related with increased risk of acute coronary syndrome. They may give an idea about various pathways involved in progression of disease and act as an important tool for predicting cardiovascular events and atherosclerosis[5]. *Secretory Phospholipase A2 group 2A* (PLA2G2A) is one of such inflammatory marker for atherosclerosis, which is a chronic inflammatory condition and *Adiponectin*, which is a hormone derived from adipose tissue is proven to have anti-inflammatory and anti-atherogenic effects. Only very few studies were available on hypothyroidism and inflammation and no study was

conducted on inflammatory markers- PLA2G2A and Adiponectin with hypothyroidism in India.

This study was done to compare the status of inflammation in hypothyroidism with these inflammatory markers and also to explore the possible relationship between hormonal replacement and the differences in risk of atherosclerosis and related markers.

Aims & Objectives

AIMS & OBJECTIVES:

PRIMARY OBJECTIVE:

To identify the possible relationship between hormone treatment and the risk of atherosclerosis in hypothyroid patients.

SECONDARY OBJECTIVE:

To explore the status of inflammation with respect to PLA2G2A and Adiponectin in hypothyroid patients.

Review of Literature

REVIEW OF LITERTURE:

The thyroid is named for the Greek word for "shield shaped", from the shape of the nearby tracheal cartilage. It was initially given importance when enlargement of thyroid gland associated with changes in the eyes and the heart was observed which is we now name as *hyperthyroidism.* Parry saw his first patient in 1786 but did not publish his findings until 1825. Graves reported the disorder in 1835 and Basedow in 1840. *Hypothyroidism* was described later, in 1874, when Gull linked atrophy of thyroid gland with the signs typical of hypothyroidism. *Myxedema* as a term was first used for a clinical syndrome which has typical thickening of subcutaneous tissue which was due to increased mucus formation in 1878 by Ord. In 1891, Murray first treated a hypothyroidism case by injecting sheep thyroid gland extract, which was found to be fully effective when given by mouth. The successful treatment of thyroid deficiency by administering thyroid extract was an important step toward modern endocrinology.

Later Gley's study on parathyroid glands in the late 19th century proved the variation of these two endocrine glands functionally. The structure of parathyroid hormone, however, was not reported until the early 1970s. Calcitonin was discovered in 1961, demonstrating that the thyroid gland produced a hormone in addition to thyroxine[6].

Anatomy:

The thyroid which is a latin word which means thyreos as shield, and eidos means form i.e. in form of shield. It contains two lobes which is connected by an isthmus. Anatomically it is situated to anterior of trachea between cricoid cartilage and suprasternal notch. Normally it's about 12-20 g with soft consistency and high vascularity. Parathyroid hormone is produced by four parathyroid glands. They are situated posterior to thyroid. On the lateral borders there is recurrent laryngeal nerves which should be noted during thyroid surgery to avoid injury resulting in vocal cord paralysis[7].

Embryological development of thyroid gland is from the floor of the primitive pharynx which develops during the third week of gestation. The gland moves along the thyroglossal duct to reach the neck. Synthesis of thyroid hormone begins normally around 11 weeks of gestation. The thyroid gland consists of numerous spherical follicles which contains follicular cells that surround secreted colloid, a proteinaceous fluid containing very high levels of thyroglobulin, precursor of thyroid hormones[8].

Physiology:

Thyroxine (T4) and triiodothyronine (T3) are the hormones formed on thyroglobulin, which is produced within thyroid cell. It is a glycoprotein. When inorganic iodide enters the follicular cell of thyroid it gets oxidized by thyroid peroxidase after which it covalently bound to tyrosine residues of thyroglobulin.

• The residues of tyrosine gets iodinated into 2 forms monoiodotyrosine (MIT) and diiodotyrosine (DIT). They undergo coupling under catalytic activity of thyroxine peroxidase to form thyronines. Two DIT forms T4 and one MIT and one DIT together form T3.

• Release of hormones into circulation is influenced by proteolysis which happens inside cells of thyroid. Hormonal transport occurs through 3 plasma proteins in bloodstream namely

- Albumin
- Thyroid binding pre-albumin and
- Thyroid binding globulin

Physiological effect mainly happens through free unbounded form of hormone which also regulate the secretion of TSH from pituitary by negative feedback mechanism.

• Among both T3 and T4, the secretion of T4 is exclusively from the gland, whereas T3 only 20% is from gland while bulk of T3 is from the T4. This T4 gets breakdown into T3, the step is catalysed by 5'-monodeiodinase. Tri iodothyronine (T3) is 5 times more active than T4.

• T4 may produce reverse T3 by the enzyme 5'-monodeiodinase, it has no important physiological activity.

• Thyroid hormone production is regulated by Thyroid Stimulating Hormone (TSH) secreted by anterior lobe of pituitary gland. Through negative feedback mechanism TSH controls the level of Free T3 and T4 in bloodstream, also helpful in influence of hypothalamic TRH. Production of

hormone and its release is mainly controlled through deiodination of T4 to T3 extracellularly. This control can be influenced by drugs, illness, nutrition and other hormones[9].

Chemistry of Thyroid Hormones:

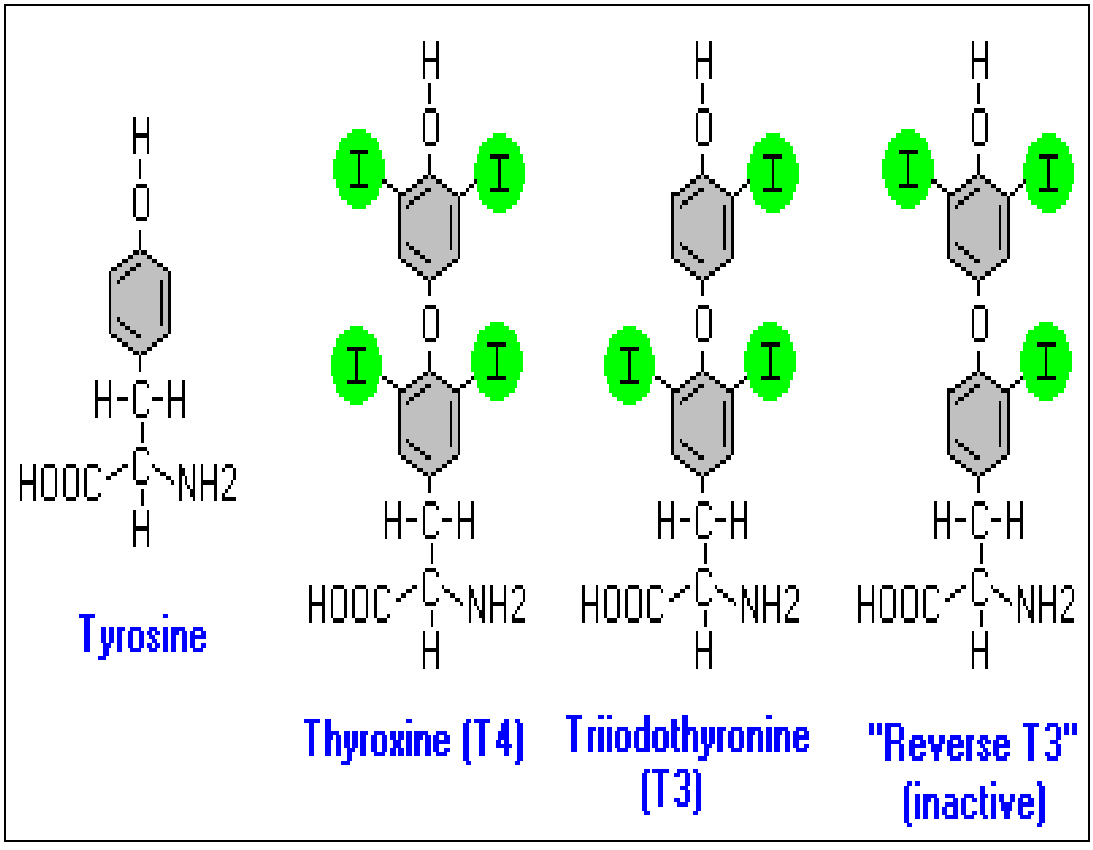
The main hormones of thyroid gland are T3 and T4. Based on the composition of hormones it was initially assumed that the activities of thyroid was due to contents in thyroxine (T4). Further studies on this aspect revealed basic preparations of hormone had more calorigenic action than that could have been due to thyroxine. Then aroused the discussion on a "second" hormone which lead to identification of T3. It was identified, secluded, and produced by Gross and Pitt-Rivers (1952). Triiodothyronine has a much higher affinity for the nuclear thyroid receptor compared with thyroxine (T4) andis much more potent biologically on a molar basis. The subsequent identification of T3 production from T4 in thyreotic humans led to the practice of effective replacement with levothyroxine alone in hypothyroidism[10].

Structure - Activity Relationships:

The structural component required for a significant degree of thyroid hormone activity has been defined. Coming to the potency the mono compound are much potent than di-substituted counterpart. Hence T3 is 5 times highly effective than thyroxine, and 3'-isopropyl-3, 5-DIT is 7 times additionally effective in its function. Substitutions at the 3, 5, 3', and 5' sites influence the conformation of the molecule. In thyroxine, structurally angle

between 2 rings at ether O_2 is120°, and along the axis they rotate freely. The presence of 3, 5 iodines restrict rotation of the two rings. In general, the binding of iodothyronines to the thyroid hormone receptors (TRs) parallels their biological potency, but additional factors can modify therapeutic potency, including affinity for plasma proteins, metabolic rate, and rate of entry into nuclei. Specific thyroid hormone transporters, like MCT8, are important in specific tissues[11].

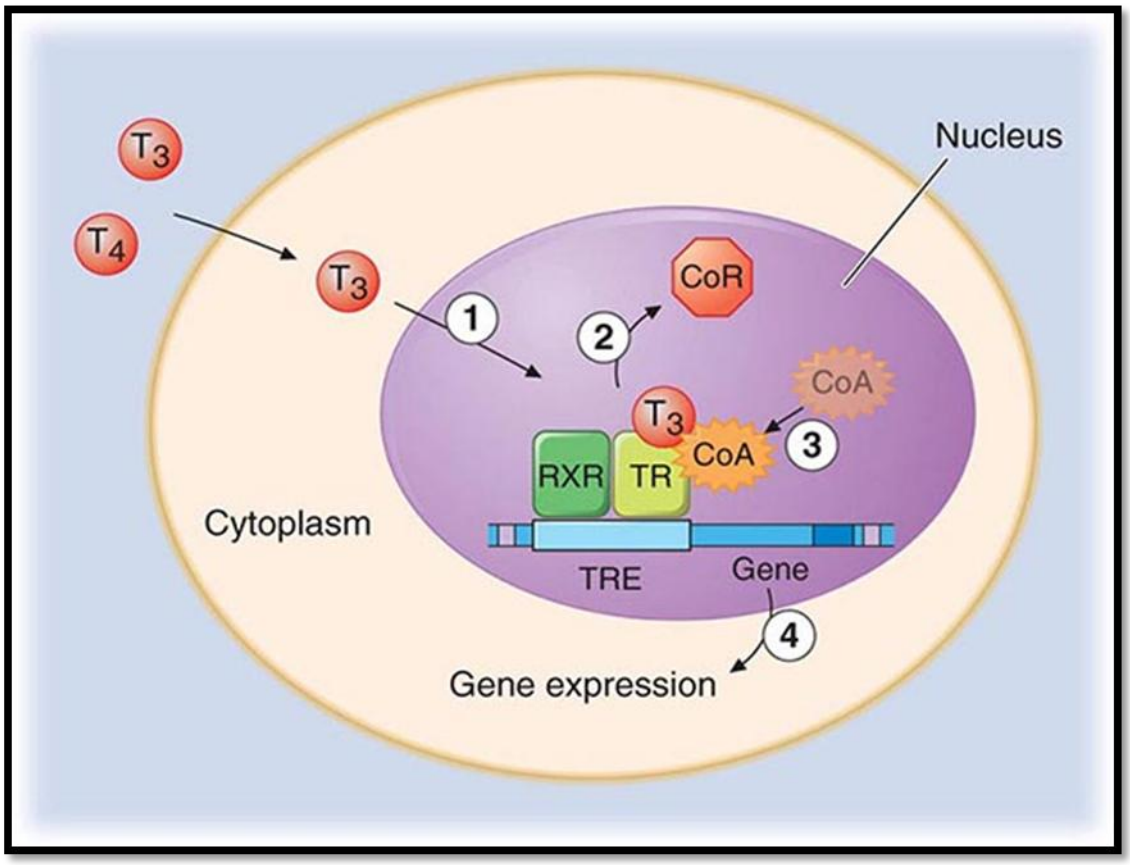
Figure 3.1 Chemistry of thyroid Hormone[6]



The stereo chemical nature of the thyroid hormones plays an important role in determining hormone activity. Many analogues of T4 are produced to confirm this SAR, also to know the hormone's antagonists and to investigate chemical structure have required activity without adverse effects. Introduction

of specific aryl methyl groups at the 3' position of triiodothyronine results in analogues that are liver-selective, cardiac sparing thyromimetics[12]. Solving the X-ray crystallographic shape of domains binding to ligands of the nuclear thyroid hormone receptors α and β has resulted in the rapid development of a range of TR isoform-selective compounds. The difference in the ligand binding domain region between TRα and TRβ is only a single amino acid (Ser 277 in TRα and Asn 331 in TRβ). GC-1, a TRβ-specific agonist, stabilizes the ligand binding domain by promoting hydrogen bonding between Asn331 and Arg282. GC-1 has a 10-fold greater affinity for TRβ, the predominant TR isoform in the liver, than TRα, the predominant TR isoform in the heart, and lowers cholesterol without stimulating the heart. Cholesterol lowering with cardiac sparing by GC-1 and other TRβ-selective agonists, however, is also the result of much higher distribution in the liver and less in the heart compared with T3. Similar compounds, such as KB-141, lower cholesterol in clinical studies[11]. Interestingly, none of these newer thyroid hormone analogs contains iodine or any halogen.

Figure 3.2 Mechanism of Thyroid Hormone Receptor Action[46]

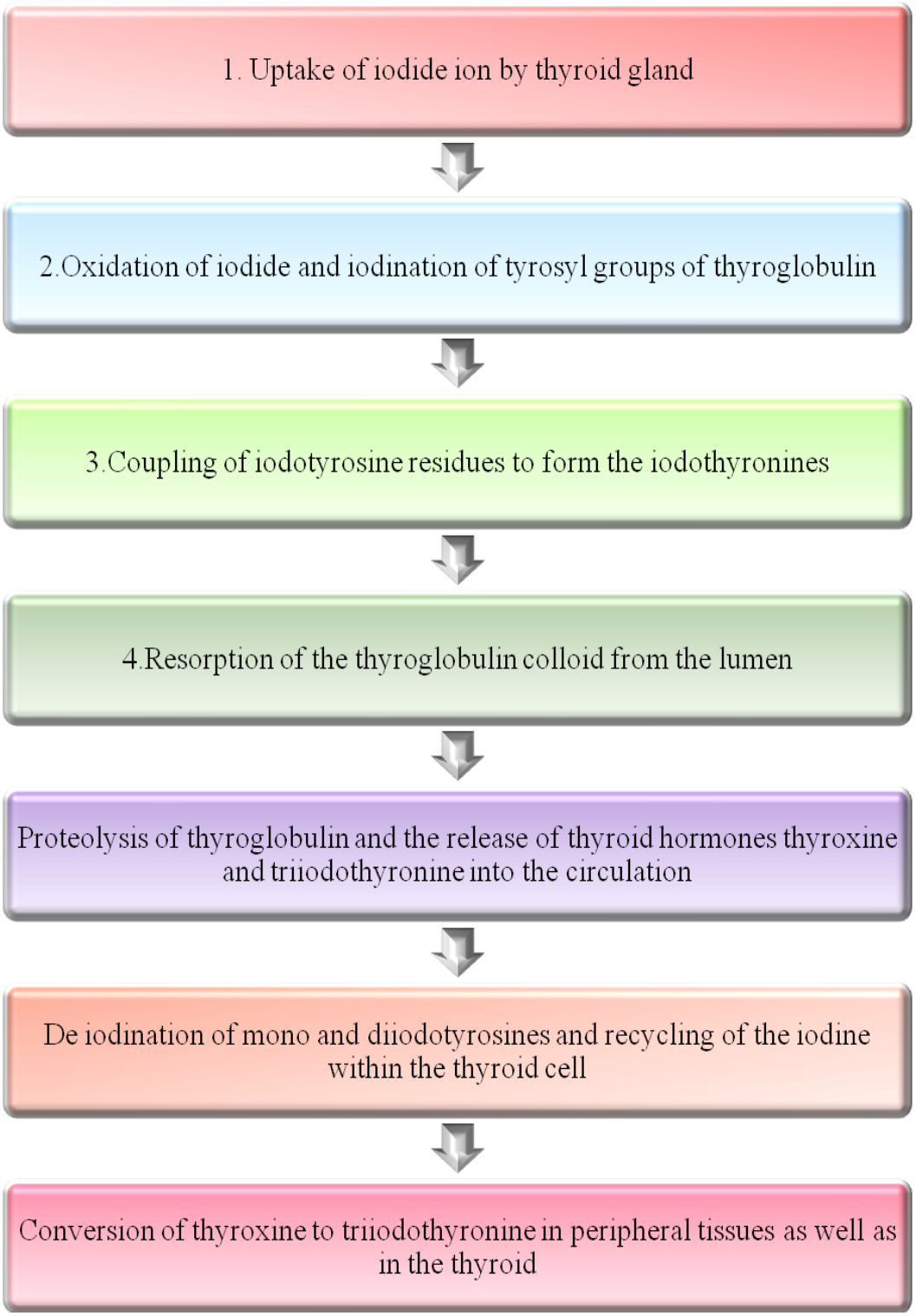


Biosynthesis of Thyroid Hormones[13-24]

The production of thyroid hormones is exclusive and complicated mechanism. The thyroid hormones are synthesized and stored in thyroglobulin. It is a protein which contains most of the colloid of thyroid follicle as residues of amino acid[13]. The gland is capable of keeping more amount of hormone by this method, and a notable part of thyroid mass constitutes extracellular thyroglobulin. It is a multifaceted glycoprotein having 2 indistinguishable units, both of 330,000 Da. Interestingly; molecular cloning has demonstrated that thyroglobulin belongs to a super family of

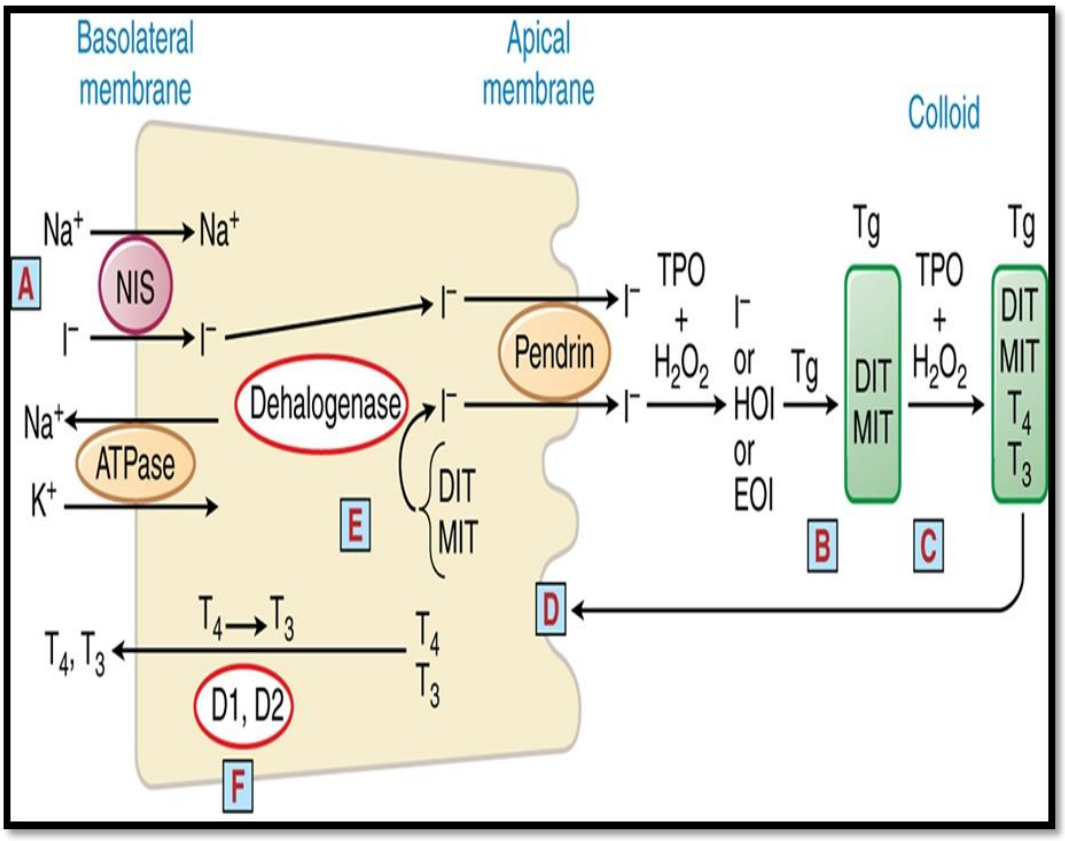
serine hydrolases, including acetylcholinesterase. The normal mechanism of synthesis, storage, release, and conversion of thyroid hormones are as follows:

Figure 3.3 Thyroid Hormone Biosynthesis and Release[6]



The above process is explained in below figure.

Figure 3.4 Thyroid Hormone Biosynthesis and Release[6]



KEY		
	METABOLIC STEP	INHIBITOR
A	Iodine transport	ClO_4^-, SCN
B	Iodination	PTU, MMI
C	Coupling	PTU, MMI
D	Colloid Resorption	Colchicine, Li^+, I^-
E	Deiodination of DIT + MIT	Dinitrotyrosine
F	Deodination of T_4	PTU

1. Iodide uptake: From diet, the iodine in form of iodide ion (I–) reaches circulation. Normally, iodine level in the plasma is too low (0.2-0.4 μg/dL; 15-30 nM), but the thyroid gland actively transports the ion via a specific membrane-bound protein, NIS. As a result this effective transport against the gradient, the ratio of thyroid to plasma iodide concentration is usually between 20 and 50 and can exceed 100 in the active gland. The iodide transport mechanism blocked by many agents like perchlorate and thiocyanate. Thyrotropin (thyroid-stimulating hormone [TSH]) stimulates NIS gene expression and promotes insertion of NIS protein into the membrane in a functional configuration.

Thus iodide uptake increased by decreased stores of thyroid iodine, and decreased by the administration of iodide by NIS protein expression. NIS has been identified in other tissues, including salivary glands, gastric mucosa, small intestine, choroid plexus, skin and perhaps placenta; all of these organs sustain a level of iodide greater than that of the blood. Iodide accumulated in the placenta and mammary glands provide adequate supplies for the foetus and infant. Accumulation of Iodine throughout the body is mediated by a single NIS gene. Individuals with congenital NIS gene mutations have absent or defective iodine concentration in all tissues known to concentrate iodine.

2. Oxidation and Iodination. Consistent with the conditions generally necessary for halogenations of aromatic rings, the iodination of tyrosine residues requires the iodinating species to be in a higher state of oxidation

than is the anion. The iodinating species is hypoiodite, either as hypoiodous acid or as an enzyme-linked species.

The oxidation of iodide to its active form is accomplished by thyroid peroxidase, a heme-containing enzyme that uses hydrogen peroxide (H_2O_2) as the oxidant. The peroxidase is membrane bound and appears to be concentrated at or near the apical surface of the thyroid cell. The reaction results in the formation of monoiodotyrosyl and diiodotyrosyl residues in thyroglobulin just prior to its extracellular storage in the lumen of the thyroid follicle. The formation of the H2O2 that serves as a substrate for the peroxidase probably occurs near its site of utilization and is stimulated by a rise in cytosolic Ca2+. The receptor to TSH is notably promiscuous in its coupling, motivating adherents of 4 G protein families including Gq, which couples to the PLC-IP3-Ca2+ pathway; thus, a Ca2+-dependent effect on H2O2 production may be a means by which TSH stimulates the organification of iodide in thyroid cells.

3. Formation of Thyroxine and Triiodothyronine from Iodotyrosines. The remaining synthetic step is the coupling of two diiodotyrosyl residues to form thyroxine or of monoiodotyrosyl and diiodotyrosyl residues to form triiodothyronine. These oxidative reactions apparently are catalyzed by the same thyroid peroxidase. The mechanism involves the enzymatic transfer of groups, perhaps as iodotyrosyl free radicals or positively charged ions, within thyroglobulin. Although many other proteins can serve as substrates for the peroxidase, none is as efficient as thyroglobulin in yielding thyroxine.

Presumably, conformation of thyroglobulin facilitates this coupling reaction. Thyroxine formation primarily occurs near the amino terminus of the protein, whereas most of the triiodotyrosine is synthesized near the carboxy terminus. The relative rates of synthetic activity at the various sites depend on the concentration of TSH and the availability of iodide. Iodine deficiency is associated with an increase in thyroidal T3 relative to T4 content.

Because triiodothyronine is the transcriptionally active iodothyronine and contains only three-fourths as much iodine, a decrease in the quantity of available iodine need have little impact on the effective amount of thyroid hormone elaborated by the gland. Although a decrease in the availability of iodide and the associated increase in the proportion of monoiodotyrosine favour the formation of triiodothyronine over thyroxine, a deficiency in diiodotyrosine ultimately can impair the formation of both compounds. Intra thyroidal and secreted T3 are also generated by the 5'-deiodination of thyroxine.

4. Resorption; 5. Proteolysis of Colloid; and, 6. Secretion of Thyroid Hormones. Because T4 and T3 are synthesized and stored within thyroglobulin, proteolysis is main chunk of secretory method. This process is started by endocytosis of colloid from the follicular lumen at the apical surface of the cell, with the participation of a thyroglobulin receptor, megalin. This "ingested" thyroglobulin appears as intracellular colloid droplets, which apparently fuse with lysosomes containing the requisite proteolytic enzymes. For hormones to be released thyroglobulin should be broken down in

particular composition as required by the hormones to be get to released. The MW of thyroglobulin is 660,000 Da, and the protein is made up of 300 carbohydrate residues and 5500 AA residues, only 2-5 of which are thyroxine; thus this is an extravagant process. TSH enhances the degradation of thyroglobulin by increasing the activity of several thiol endopeptidases of the lysosomes. Endopeptidases selectively cleave thyroglobulin, yielding hormone-containing intermediates that subsequently are processed by exopeptidases. The liberated hormones then exit the cell, presumably at its basal membrane. When the TG is hydrolyzed, MIT & DIT also released but usually they doesn't leave from gland; rather, gets selectively metabolized and the iodine, liberated as I–, is reincorporated into protein. The iodotyrosine deiodinase enzyme, DHAL1, is essential for conserving iodine and mutations of this gene identified in several kindred's are associated with goitrous hypothyroidism and cognitive deficit. When there is intensive activation of proteolysis by TSH these iodine gets reused, rest reaches blood, sometime along with iodotyrosines.

7. Thyroid Hormone Metabolism and Conversion of Thyroxine to Triiodothyronine in Peripheral Tissues. The normal production of thyroxine is estimated to range between 80 and 100μg/day; that of triiodothyronine is between 30 and 40μg. Although triiodothyronine is secreted by the thyroid, metabolism of thyroxine by 5', or outer ring, deiodination in the peripheral tissues accounts for ~80% of circulating triiodothyronine. In contrast, removal of the iodine on position 5 of the inner ring produces the metabolically

inactive 3, 3', 5'-triiodothyronine (reverse T3, rT3). Under normal conditions, ~40% of T4 is converted to each of T3 and rT3, and ~20% is metabolized by other pathways, such as glucuronidation. Normal level of T4 in plasma is 4.5-11 μg/dL; those of T3 are ~1/100 of that (60-180 ng/dL). The types 1 and 2 deiodinases (D1, D2) convert thyroxine to triiodothyronine. These enzymes contribute approximately equally to the plasma T3 in rats, but it has not been possible to determine their relative contributions in humans. D1 is expressed primarily in the liver and kidney, and also in the thyroid and pituitary. It is up regulated in hyperthyroidism and down regulated in hypothyroidism. A clinically important feature of D1 is its inhibition by the anti-thyroid drug *propylthiouracil*. D1 is localized to the plasma membrane, and the T3 it produces equilibrates rapidly with the plasma. D2 is present principally in CNS and adipose tissue mainly brown, also in the thyroid, and at very low levels in other organs such as skeletal muscle. The activity of D2 is unaffected by propylthiouracil. D2 localizes to the endoplasmic reticulum, which facilitates access of D2-generated T3 to the nucleus. Hence organs that express D2 tend to use the locally generated T3 to increase the occupancy of nuclear T3 receptors, and D2-generated T3 equilibrates slowly with the plasma. D2 is dynamically regulated by its substrate, thyroxine, such that elevated levels of the enzyme are found in hypothyroidism and suppressed levels is found in hyperthyroidism. Thus, D2 autoregulates the intracellular supply of triiodothyronine in organs in which it is expressed. Inner ring- or 5-deiodination, the main inactivating pathway of T3 metabolism, is catalyzed

mainly by the type 3 deiodinase (D3), and to some extent also by D1. D3 is found at highest levels in the CNS and placenta, and it also is expressed in skin and uterus. It is highly expressed in hemangiomas. D3 can be induced at sites of inflammation, and at least in an animal model this can result in local hypothyroidism.

D2 and D3 also play important roles in regulating local T3 levels during development, during which thyroid hormone tends to promote differentiation and decrease proliferation. Examples include chondrocyte development and bone differentiation, and auditory (cochlea) development. In the brain, D2 is expressed in glial cells and D3 in neurons.

Because T3 receptors are enriched in neurons, the current model is that glial cells convert T4 to T3, which is then exported and taken up by neurons where it activates T3 receptors, with subsequent degradation by D3 and termination of the T3 effect. There is a growing recognition of circumstances where D2 and D3 regulate local thyroid hormone levels independent of the plasma T4 and T3 concentrations. The three deiodinases contain the rare amino acid selenocysteine in their active sites. Incorporation of selenocysteine into the growing peptide chain is a complex process involving multiple proteins. Mutations in one such protein, SECIS binding protein 2, are associated with abnormal circulating thyroid hormone levels.

Transport of T3 and T4 Hormones in the Blood:

Iodine is normally present in several forms, with 95% as organic iodine and ~5% as iodide. Most organic iodine is thyroxine (90-95%); triiodothyronine represents a relatively minor fraction (~5%). The hormones are moved into circulation by a non-covalent solid link with plasma proteins[22].TBG is an important transporter of T3 & T4. It is an acidic glycoprotein with a molecular mass of ~63,000 Da that binds one molecule of T4 per molecule of protein with a very high affinity (the equilibration dissociation constant, Kd, is ~10^{-10} M). T3 is bound less avidly. Thyroxine, but not triiodothyronine, also is bound by transthyretin (thyroxine-binding prealbumin), a retinol-binding protein. This protein is present in higher concentration than is TBG and primarily binds thyroxine with an equilibrium dissociation constant ~10^{-7} M. Transthyretin has four apparently identical subunits but only a single high-affinity binding site. Albumin also can bind thyroxine when the more avid carriers are saturated, but it is difficult to estimate its quantitative or physiological importance except in *familial dysalbuminemic hyperthyroxinemia.* It is an AD disorder with increased affinity of albumin for thyroxine, the reason behind this is point mutation in gene encoding albumin. There are a number of genetic defects of thyroid-binding globulin[22, 23], but these individuals generally have normal circulating free thyroid hormones and serum TSH, and they are euthyroid. The free (unbound) hormone is a small percentage (~0.03% of thyroxine and ~0.3% of triiodothyronine) of the total hormone in plasma. The differential binding

affinities for serum proteins also contribute to establishing the 10- to 100-fold differences in circulating hormone concentrations and half-lives of T4 and T3.

Essential to understanding the regulation of thyroid function is the “free hormone” concept: Only the unbound hormone has metabolic activity. Thus, because of the high degree of binding of thyroid hormones to plasma proteins, changes of plasma level of hormone may affect the affinity to their proteins. Certain drugs and a variety of pathological and physiological conditions, such as the changes in circulating concentrations of estrogens during pregnancy or during the administration of oral estrogens, can alter both the binding of thyroid hormones to proteins and the amounts of these proteins. However, because the pituitary responds to and regulates circulating free hormone levels, minimal changes in free hormone concentrations are seen. Therefore, laboratory tests that measure only total hormone levels can be misleading.

Degradation and Excretion: Thyroxine is eliminated slowly from the body, with a t1/2 of 6-8 days. In patient with hyperthyroidism, the t1/2 is shortened to 3-4 days, whereas in patient with hypothyroidism it may be 9-10 days. These changes presumably reflect altered rates of metabolism of the hormone. In conditions associated with increased binding to TBG, such as pregnancy, clearance is retarded. The increase in TBG is due to the oestrogen-induced increase in the sialic acid content of the synthesized TBG, resulting in decreased TBG clearance. The opposite effect is observed when there is reduced protein binding of thyroid hormones or when binding to protein is

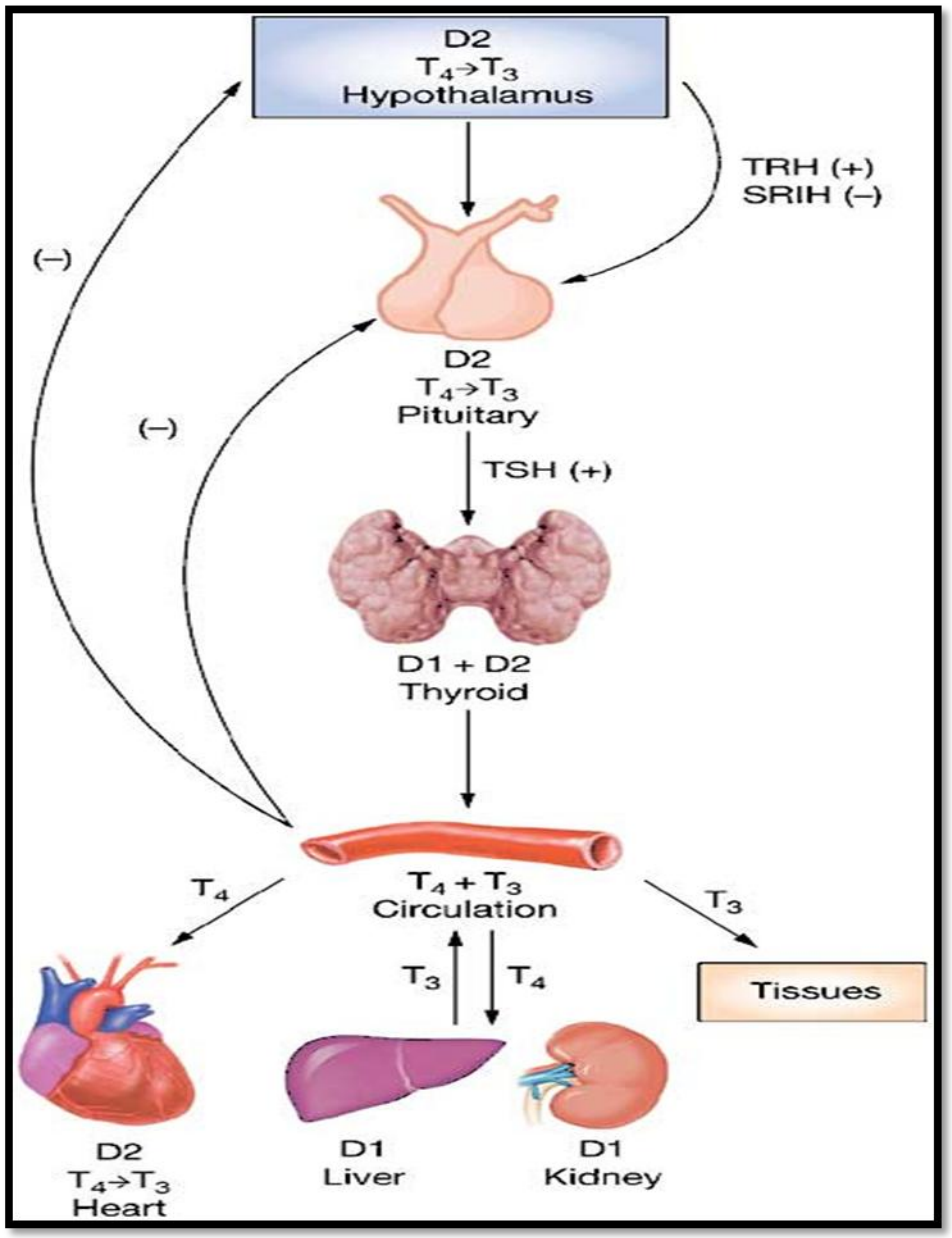
inhibited by certain drugs. T3, which is less avidly bound to protein, has a t1/2 of around 1 day.

The important site of degradation of hormones is liver; T4 & T3 undergo glucronide conjugation and also sulfate conjugation ending up in biliary excretion. Some thyroid hormone is liberated by hydrolysis of the conjugates in the intestine and reabsorbed. A portion of hormone which has undergone conjugation is hydrolysed in colon and faecal excretion occurs.

As discussed earlier, the important pathways of metabolism of T4 is deiodination into T3 or reverse T3. Triiodothyronine and reverse T3 are deiodinated to three different DIT, they are then deiodinated to two MIT, inactive forms which are usual constituents of human plasma.

Regulation of Thyroid Function: Thyrotropin or TSH is a glycoprotein hormone with α and β subunits analogous to those of the gonadotropins. Normally TSH is released in circadian rhythm and pulsate pattern; with peak levels during sleep. Its secretion is mainly regulated by hypothalamus which releases TRH and in relevant to thyroid profile in blood stream. Excess hormone present inhibits transcription of gene for TRH and those for thyrotropin α and β subunit, it suppresses TSH secretion causing regression of thyroid[24]. When there is below normal level of hormone, the thyroid spontaneously increases the secretion of TSH. Additional mechanisms mediating the action of T3 and T4 on TSH seems to be due to reduction in TRH secretion by the hypothalamus and a reduction in the number of TRH receptors on pituitary cells.

Fig 3.5 Regulation of thyroid hormone secretion[47].



Thyrotropin-Releasing Hormone: TRH stimulates the release of preformed TSH from secretory granules and also stimulates the subsequent synthesis of both α and β subunits of TSH. Somatostatin and glucocorticoids

at therapeutic doses inhibit TRH-stimulated secretion of TSH. The mature hormone is produced from protein that has 6 copies of the tripeptide attached to dibasic residues [25].

TRH also has been localized in the CNS in the cerebral cortex, circumventricular structures, neurohypophysis, pineal gland, and spinal cord. These findings, as well as its localization in nerve endings, suggest that TRH may act as a neurotransmitter or neuromodulator outside of the hypothalamus. Administration of TRH to animals produces CNS-mediated effects on behavior, thermoregulation, autonomic tone, and cardiovascular function, including increases in blood pressure and heart rate.

TRH also has been identified in pancreatic islets, heart, testis, and parts of the gastrointestinal tract. Its physiological role in these sites is not known. Two TRH receptors have now been identified, TRH-R1 and TRH-R2, as well as selective analogs for these receptors[25]. TRH analogs with CNS activity, but without other hormonal actions, are being developed for therapeutic applications. TRH is no longer available in the U.S[26].

Actions of Thyroid-Stimulating Hormone: On administration of TSH in animals, the first measurable consequence on thyroid hormone metabolism is increased secretion, which is detectable within minutes. All phases of hormone synthesis and release are eventually stimulated: iodide uptake and organification, hormone synthesis, endocytosis, and proteolysis of colloid.

There is increased vascularity of the gland and hypertrophy and hyperplasia of thyroid cells.

These effects follow the attachment of TSH and its receptor on thyroid cell plasma membrane. The TSH receptor is a GPCR that is structurally similar to the receptors for luteinizing hormone and follicle-stimulating hormone[26]. These receptors share significant amino acid homology and have large extracellular domains that are involved in hormone binding. Binding of TSH to its receptor stimulates the Gs-adenylyl cyclase–cyclicAMP pathway. Higher concentrations of TSH activate the Gq-PLC pathway. Both the adenylyl cyclase and the phospholipase-C signaling pathways appear to mediate action of TSH on functioning of thyroid in humans[27]. Germline mutations can present as congenital, non autoimmune hypothyroidism or as AD toxic thyroid hyperplasia[28]. Transmutations of the TSH-R can cause gestational hyperthyroidism due to a hypersensitivity of the receptor to HCG[29]. Somatic mutations that result in stimulation of the receptor are associated with increased activity of thyroid adenomas[30]. Finally, resistance to TSH has been described, both in families with mutant TSH receptors[31.]

Relation of Iodine with Thyroid Function: Sufficient iodine intake is required for routine functioning of thyroid, when it is less there will be abnormality in functioning of thyroid and elevation in TSH levels results in enlargement of thyroid. As a result there will be increased extraction of thyroid, developing an iodine gradient that may be 10 times normal; in mild to moderate cases through this mechanism the deficiency is somewhat balanced

but in severe deficiency as this balance cannot be maintained, cretinism in children and hypothyroidism in adult occurs[32].

Regions of iodine deficiency exist in Central and South America, Africa, Europe, Southeast Asia, and China. In the U.S., recommended daily allowances for iodine range from 90-120 μg for children, 150μg for adults, 220 μg for pregnancy, and 290 μg for lactation[33]. Vegetables, meat, and poultry contain minimal amounts of iodine, whereas dairy products and fish are relatively high in iodine. The success of many experiments treatment led to the adoption of iodine prophylaxis and therapy in many regions throughout the world where iodine-deficiency goiter was endemic[20,33].

The most practical method in substituting iodide or iodate is by table salt; iodate is now preferred. The use of iodized salt is required by law in some countries, but in others such as the U.S., the use is optional. In the U.S., iodized salt provides 100 μg of iodine per gram. Although the U.S. population remains iodine sufficient, iodine intake has steadily decreased over the last 20 years[34,35]. The most recent data indicate that iodine intake has stabilized, although pregnant women remain a susceptible population for iodine insufficiency[35]. Other vehicles for supplying iodine to large populations who are iodine deficient include oral or intramuscular injection of iodized oil, iodized drinking water supplies, iodized irrigation systems, and iodized animal feed[36,37].

Actions of Thyroid Hormones:

Classical Nuclear-Mediated Effects: It is mediated largely by attachment of T3 to TRs belonging to nuclear superfamily[38,39]. They also consist of receptors for,

- **Vitamin D**
- **Steroid hormones**
- **Fatty acids**
- **Retinoic acid**
- **Bile acid**

The nuclear receptor superfamily also includes a number of "orphan receptors" that have no known ligands and may be regulated by post translational modifications or other events. The TRs have the classic nuclear receptor structure[39].

T3 binds to TRs with 10-fold greater affinity than does T4, and T4 is not thought to be biologically active in normal physiology. TRs bind to specific DNA sequences (TREs) in the promoter/regulatory regions. The transcription of most target genes is repressed by unliganded TRs and induced following the binding of T3. The mechanisms of these effects have been well studied[39]. In the unliganded state, the TR ligand binding domain interacts with a co-repressor complex that includes histone deacetylases and other proteins. The binding of T3 causes replacement of the co-repressor complex by a co-activator complex that includes histone acetyltransferases, methyltransferases, and other proteins. The actual situation is very complex and probably involves

cyclical recruitment of multiple protein complexes to the target gene, leading to cyclical transcription despite the constant presence of T3[40,41]. Rest are negatively regulated by T3. The mechanism is not well defined, but these genes tend to be induced by the unliganded TR in addition to being repressed by T3.

The TRs are the cellular homologs of the avian retroviral oncoprotein c-*erb*A. There are two genes that encode TRs, *THRA* and *THRB. THRA* encodes the receptor TRα1. The biological function of TRα1 is inferred largely through studies of genetically modified mice and cell culture experiments because human mutations in *THRA* have not been described. Although TRα1 is expressed in most cell types, studies in knockout mice demonstrate that the major specific roles for this isoform are in the regulation of heart rate, body temperature, skeletal muscle function, and the development of bone and small intestine[41]. Alternative splicing of the TRα primary transcript results in the production of TRα2, which does not bind T3 because it lacks part of the ligand binding domain (LBD). TRα2 is expressed ubiquitously and its function is unknown, although there is some evidence that it may inhibit T3 action. A cryptic promoter within intron 7 of *THRA* drives the production of small proteins that contain only a portion of the TRα LBD. These non-hormone-binding proteins appear to play a role, along with TRα1, in GI development.

The *THRB* gene has two promoters that lead to the production of TRβ1 and TRβ2. These receptors have unique amino terminal domains but

otherwise are identical. TRβ1 is ubiquitous, whereas TRβ2 has a highly restricted pattern of expression. Mutations in *THRB* cause the syndrome of resistance to thyroid hormone. Studies in these patients, as well as in genetically modified mice and in cell culture, demonstrate a specific role for TRβ1 in liver metabolism (including the hypocholesterolemic effect of T3), and for TRβ2 in the negative feedback by T3 on hypothalamic TRH and pituitary TSH[43]. TRβ2 also is important in the development of cones in the retina and in inner ear development.

Non-genomic Effects of Thyroid Hormone[44]: Although nuclear receptors were classically described as being DNA-binding transcription factors, it is now clear that these proteins also can be found outside the nucleus where they can exert biological effects via rapid non genomic mechanisms[48]. TRs associate in a T3-dependent manner with the p85α subunit of phosphatidyl inositol 3-kinase (PI3K), which results in the phosphorylation and activation ofPKB/Akt[45]. The PI3K/Akt pathway has broad effects on cellular metabolism. For example, it stimulates NO production by endothelial cells, which leads to vasodilation. Hence, T3 administration causes rapid vasodilation. There also is evidence for non-genomic actions of thyroid hormone via a plasma membrane receptor within integrin αVβ3. This putative receptor binds extracellular T4 in preference to T3, resulting in activation of MAP kinase. However, the importance of non-genomic actions in thyroid hormone physiology and pathophysiology remains uncertain.

Effects of Thyroid Hormone Metabolites: 3-Iodothyronamine and thyronamine, naturally occurring metabolites of T4, are ligands for the GPCR trace amine associated receptor 1 (TAAR1)[49]. Administration of 3-iodothyronamine or thyronamine to mice causes hypothermia and bradycardia, presumably through activation of TAAR1 and Gs. 3-Iodothyronamine activation of TAAR1 also induces insulin secretion in the MIN6 cell line[50]. Interestingly, 3-iodothyronamine also binds to the α2A adrenergic receptor, leading to decreased insulin secretion from mouse islets via a Gi protein coupled mechanism. The importance of these effects in human physiology and disease remains to be determined.

Major Clinical Effects of Thyroid Hormones:

Growth and Development: Perhaps the most dramatic example of thyroid hormone action is amphibian metamorphosis, in which the tadpole is almost magically transformed by triiodothyronine into adult frog. They also have a main part in development of brain[51,52]. If such hormones are not available during the first 6 months after birth it may cause cretinism with mental abnormalities, abnormal development of brain structure will also ensue. The main reasons for these changes are problem in projection of axon, synapticity and problem in migration of neuron. So it's better to give a minimal dose for two wks at least to high risk babies to avoid any such abnormalities.

The mechanisms by which thyroid hormone promotes brain development are incompletely understood. Surprisingly, mice with genetic deletions of TRα,TRβ, or both have essentially normal brain development[53].

Equally surprisingly, genetic deletion of TRα1 protects mice from the toxic effects of hypothyroidism on brain development. Perhaps it is the effects of unliganded TRs that result in impaired brain development, such that either T3 or the absence of TRs is protective. T3 induces the expression of a number of genes that could plausibly be important in normal brain development, but the exact roles of these specific gene inductions are not known[51].

The T3-dependent expression of many proteins appears to be merely delayed in the hypothyroid animal; normal levels are eventually achieved.RC3/neurogranin, a protein involved in synaptic plasticity, also is induced by T 3. Through its induction of the transcription factor BTEB (basic transcription element binding protein), T3 expands a network of secondary genes in brain development.

The actions of thyroid hormones on protein synthesis and enzymatic activity are not limited to the brain, and in fact most tissues are affected by the administration of thyroid hormone or by its deficiency. The extensive defects in growth and development in cretins vividly illustrate the pervasive effects of thyroid hormones in normal individuals.

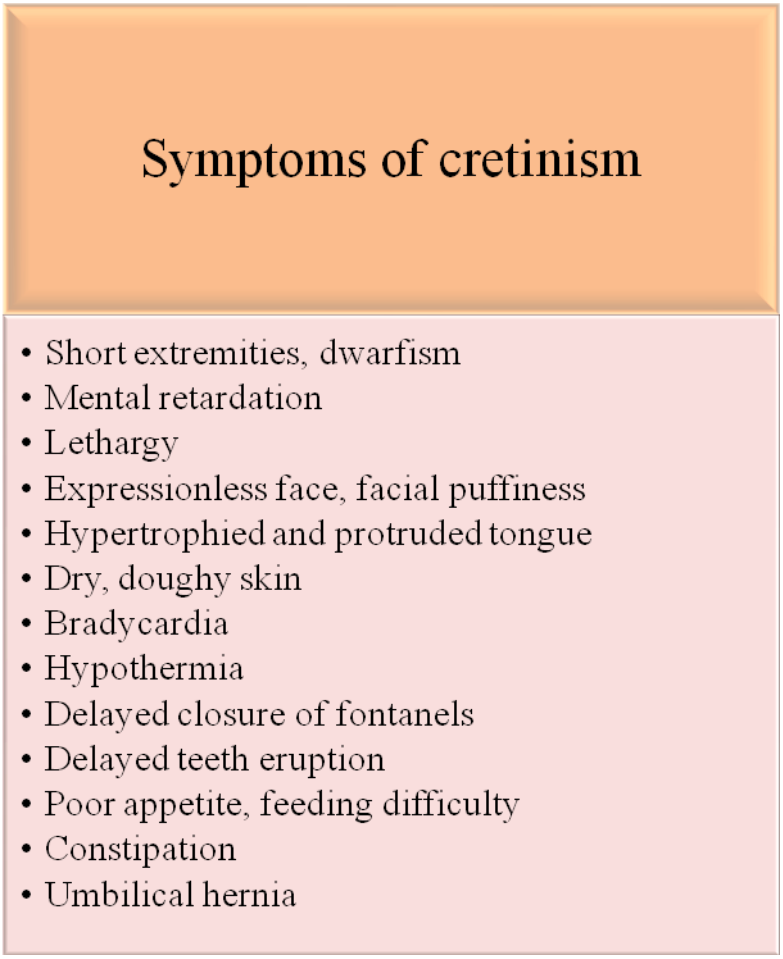
Cretinism is of two types:

1. Sporadic
2. Endemic

Endemic cretinism is mostly seen in places where endemic goiter is present, main cause is severe deficiency of iodine.

Sporadic type is due to failure of normal development of thyroid or defective synthesis of hormone. In case of synthesis defect goiter may be present.

Figure 3.6 Symptoms of Cretinism



For an active management early diagnosis should be made before appearance of clear signs and symptoms. In region of endemic cretinism, for pregnant women earlier iodine replacement should be started so that when it is given up to 2nd trimester there will better development of brain and also psychologically for the infants[54,99]. Concentrations of TSH and thyroxine are

measured in blood from the umbilical cord or from a heel stick. The incidence of congenital dysfunction of the thyroid is 1 per 4000 births.

Thermogenic Effects: Thyroid hormone is necessary for both obligatory thermogenesis (the heat resulting from vital processes) as well as for facultative or adaptive thermogenesis[55]. Obligatory thermogenesis is the result of T3 making most biological processes thermodynamically less efficient for the sake of producing heat. Multiple mechanisms underlie the stimulation of obligatory thermogenesis, but the pathways involved and their quantitative contributions have yet to be fully defined. The ability of T3 to induce the skeletal muscle Ca++-dependent ATPase (SERCA1) contributes to thermogenesis by stimulating the cycling of calcium between cytosol and sarcoplasmic reticulum.

Metabolic “futile cycling" (e.g., lipogenesis-lipolysis) has been considered a mechanism to produce heat, but best estimates indicate the contribution is minor. Other than in brown adipose tissue, there is no evidence that a major thermogenic mechanism is uncoupling of phosphorylation, although some groups still believe that T3 can increase the proton leak through the inner mitochondrial membrane. Regardless of the mechanism, thermogenesis is highly sensitive to thyroid hormone around the physiological range because small changes in L-thyroxine replacement doses may significantly alter resting energy expenditure in the hypothyroid patient. The discovery of type 2 deiodinase in brown fat and its activation by the sympathetic nervous system showed that thyroid hormone also is important

for facultative thermogenesis. The ability of T3 to stimulate thermogenesis has evolved along with ancillary effects to support this thermogenic action, such as the stimulation of appetite and lipogenesis.

Cardiovascular Effects: Obvious changes are seen in heart and related structure in both hyper- and hypothyroidism[56]. Hyperthyroid patients have tachycardia, augmented SV, CI greater than before, LVH, amplified PP and diminution in PVR. Hyperthyroidism is a relatively common cause of atrial fibrillation. Hypothyroid patients have decrease in HR and cardiac index, pericardial effusion, augmented PVR, diminuted PP and increase in MAP.

Triiodothyronine directly regulates myocardial gene expression primarily through TRα1, which is expressed at a higher level in cardiomyocytes than TRβ. T3 shortens diastolic relaxation (lusitropic effect) by inducing expression of the sarcoplasmic reticulum ATPase SERCa2 and decreasing phospholamban, a SERCa2 inhibitor. T3 increases the force of myocardial contraction (inotropic effect) in part by inducing expression of the ryanodine channel, the calcium channel of the sarcoplasmic reticulum. T3 induces the gene encoding the myosin heavy chain (MHC) α isoform and decreases expression of the MHC β gene. Because MHCα endows the myosin holoenzyme with greater ATPase activity, this is one mechanism by which T3 enhances the velocity of contraction. The chronotropic effect of T3 is mediated by increases in the pacemaker ion current if in the sinoatrial node. Several proteins that comprise the If channel are induced by T3, includingHCN2 and HCN4. The vasodilation effect on SM by direct action of

T3 is sometime present, which may contribute to the decreased systemic vascular resistance and increased cardiac output of hyperthyroidism.

Metabolic Effect: They also increases presence of low-density lipoprotein LDL-R and the also metabolises bile acids from cholesterol, hence increase in cholesterol levels is important feature of hypothyroidism. Thyroid hormone has complex effects on carbohydrate metabolism[57]. Thyrotoxicosis is an insulin-resistant state. In addition, the rate of glucose absorption from the gut is increased. Compensatory increases in insulin secretion result in hyperinsulinemia. There may be impaired glucose tolerance or even clinical diabetes, but most hyperthyroid patients are euglycemic. However, diabetic patients already on insulin may have increased insulin requirements in the setting of hyperthyroidism. On the other hand, there will be decrease in glucose absorption, along with reduction in secretion of insulin. Glucose metabolism generally is not affected in a clinically significant manner in non-diabetic patients, although insulin requirements decrease in the hypothyroid patient with diabetes.

Pathophysiology of hypothyroidism:

Aetiology of 1º Hypothyroidism includes,

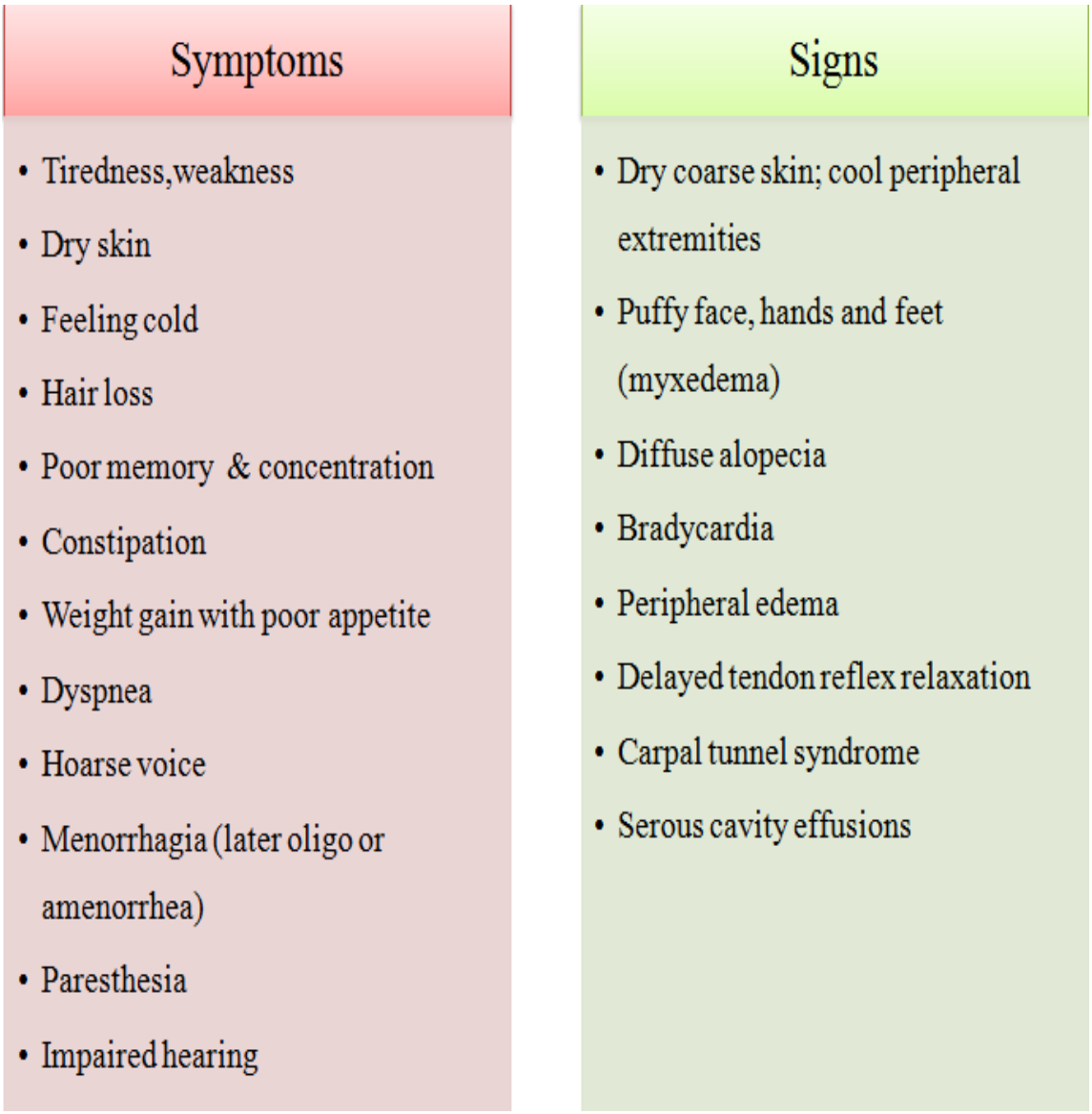
1. Hashimoto's disease - an autoimmune condition
2. Iatrogenic hypothyroidism
3. Iodine deficiency

4. Enzyme defects
5. Hypoplasia of Thyroid gland
6. Goitrogens
7. Family H/O hypothyroidism[100]

Aetiology of 2º Hypothyroidism includes,

1. Surgery
2. Tumours
3. Radiotherapy
4. TB
5. Autoimmune disease
6. Sheehan's syndrome
7. Histiocytosis

Figure 3.7 Clinical Features of Hypothyroidism[46]



Diagnosis:

• Thyroid profile test

- Serum TSH - increased
- FT4 - sometimes normal initially
- As the disease progresses, FT4 reduces less than normal range

- T3 is often within normal range level.
- Anti-peroxidase antibodies and anti-thyroglobulin antibodies are likely to be elevated.

• Secondary hypothyroidism should be suspected in a patient with decreased levels of T4 and inappropriately normal or low TSH levels.

Treatment of hypothyroidism:

• Drug of choice –Levothyroxine

Advantages:

- Stable
- Cheap
- No antigenicity
- Good potency.

- Required dose based on body weight 1.6 µg/kg46,101.
- For young patients with concomitant chronic illness
- Patients > 45 yrs without known cardiac disease

Starting dose is 50µg which can be raised up to 100µg daily

• For patients > 45 yrs with cardiac condition

- Starting dose daily – 25 mcg
- Raised by 25 mcg every month

• Adults – maintenance dose - 125µg/day

• Levothyroxine can be given for pts with subclinical cases and elevated levels of Thyroid stimulating antibodies.

•During Pregnancy - Levothyroxine

Main aim is to reduce TSH to 1 mIU/L

- Drug influence on levothyroxine
    - Cholestyramine
    - $CaCO_3$
    - Al_2OH
    - $FeSO_4$ and
    - Dietary fibre supplements

These drugs have influence on absorption.

• Drugs raising nondeiodinative T4 clearance

- ✓ Rifampin
- ✓ Carbamazepine
- ✓ Phenytoin

Amiodarone blocks the conversion of T4 to T3

Thyroid, USP

- ❖ Extracted from thyroid of sheep, beef
- ❖ Antigenicity present
- ❖ Bioequivalence is absent

Thyroglobulin

- ❖ Purified extract from hog-gland
- ❖ Ratio of T4:T3 is 2.5:1
- ❖ Not used much

.

Liothyronine

- Synthetic form of T3
- Good potency
- Higher cardiotoxicity
- Costly
- Follow up is difficult

Liotrix

- Synthetic form
- T4:T3 ratio is 4:1
- Stable chemically
- Steady potency
- Costly

General effects due to hormone replacement:

- Cardiac failure
- Angina pectoris
- Myocardial infarction
- Allergic reactions
- Reduced Bone mineral density

Treatment of myxoedema coma:

• Initial treatment

Levothyroxine

Route: IV bolus, 300 µg to 500 µg

• Followed by Liothyronine IV

• Hydrocortisone IV100 mg/TDS until adrenal suppression is ruled out.

Recovery expected within 24 hours.

• Maintenance dose is 75µg to 100 µg IV

• Then continued by oral therapy

• Supportive therapy may be required

• Maintain BP, blood sugar, temperature, ventilation

Evaluation of therapeutic outcomes:

• Serum TSH levels -Sensitive and specific

Best value for dose adjustment.

Normally values gets corrected by 6 wks

• Every six wks - TSH and T4 monitoring to be done.

Until normal reference range reaches.

If TSH is high - dose is not enough.

Serum T4 level useful in-

1. Spotting malabsorption
2. Non compliance
3. Bioequivalence abnormalities

Serum TSH - identifies noncompliance.

• If deficiency due to failure of pituitary or hypothalamus only way is replacement by throxine and monitoring it.

Atherosclerosis:

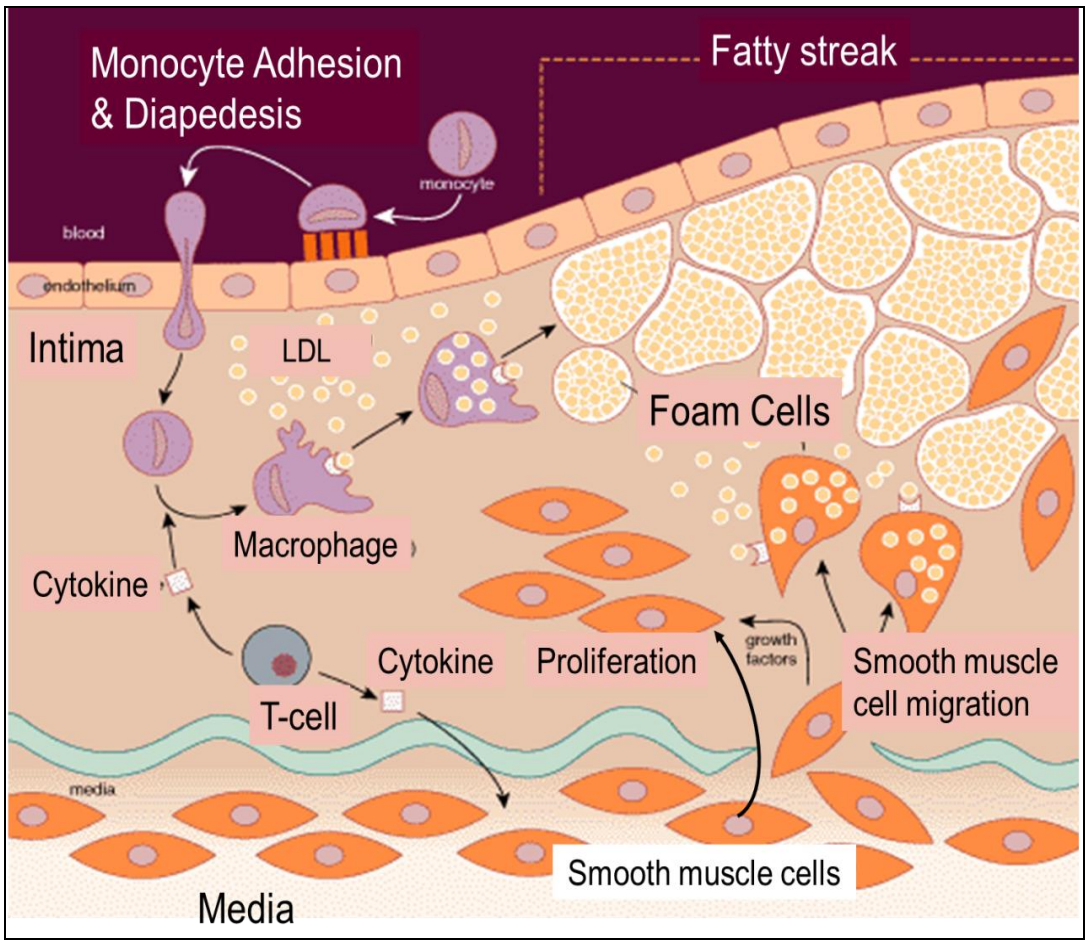
Atherosclerosis is mainly due to increased deposit of lipid plaques in the bigger vessels and its wall is one of the common causes of incidence of cardiovascular disease. The global occurrence is 11%, and occurrence standardized by age is 9.0%[58, 59]. It is more in patients with impaired glucose tolerance and DM compared to healthy group. In Indians there is basically low HDL level resulting in a premature CAD in Asian ethnicity. Similarly LDL is also on higher side in CAD compared to healthy people. But altogether even with minimal elevation of LDL associated with moderate increase in LDL/HDL and low HDL and also increase in TC:HDL ratio may lead to atherosclerosis.

Though pathophysiology is yet to be assessed in detail atherosclerosis is thought to be stimulated by combination of multiple factors like condition of blood flow, deposition of lipoprotein in sub intima and its variation, and malfunction of endothelium. Disease progression is related to chronic inflammatory response which causes fibrosis, necrosis of tissue and finally thrombosis, since it's a chronic disease it is more important to observe the whole pathogenesis on trot which need a longer time of study. Dysfunction of endothelium is one of first and important indicator of atherosclerosis, and is commonly observed in prospective clinical studies and they appear even before clinical manifestation of the disease.

Atherosclerostic plaque formation is the important and primary cause for CAD and ACS. Dysfunction of endothelium leads to the generation of fatty

streaks in the walls of the coronary arteries and then formation of atherosclerotic plaques. The cardiovascular complications develop mainly due to rupture, erosion or fissuring of an unstable plaque. The susceptible plaques usually have a thin fibrous cap, high fatty core, large number of inflammatory cells like macrophages and lymphocytes[60].

Figure 3.8 Chronic inflammation and atherosclerosis[61]

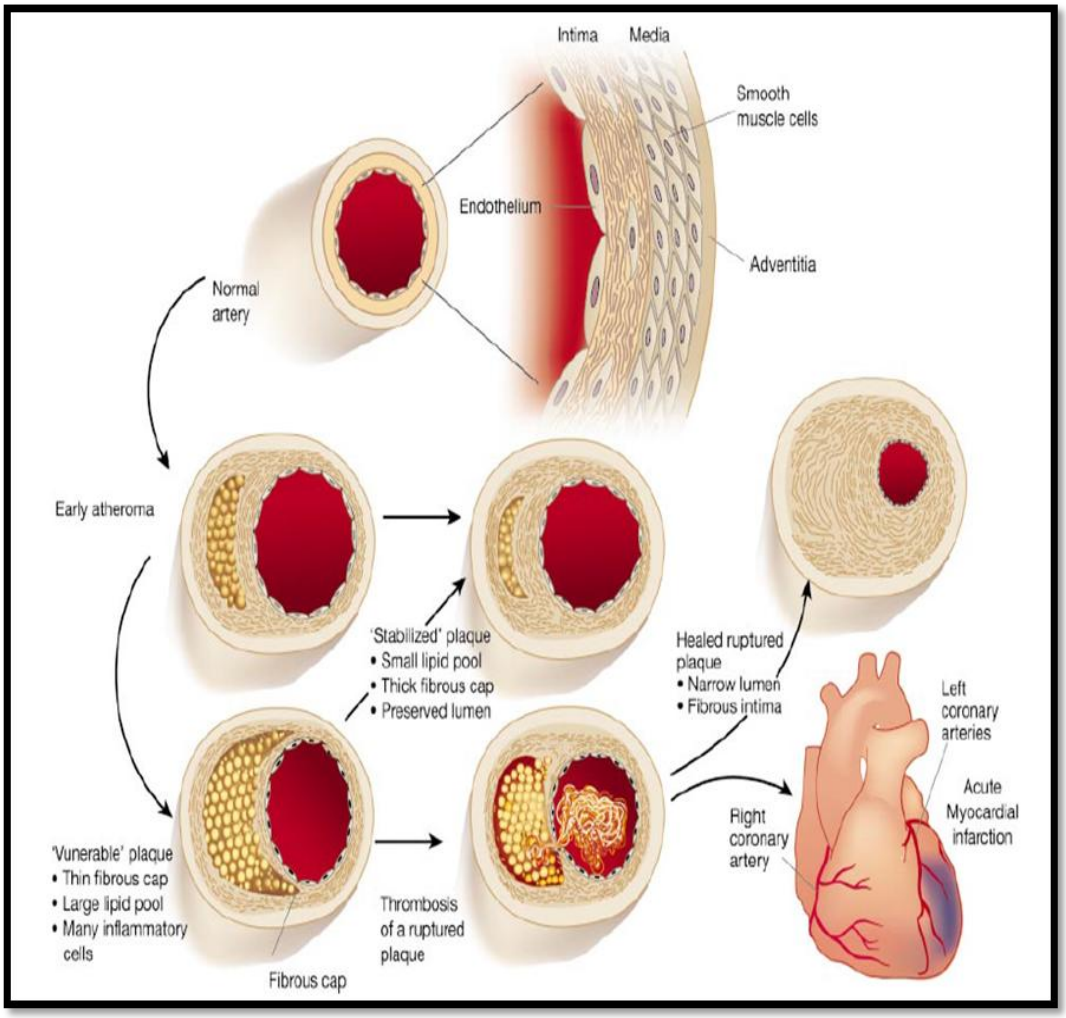


Evolution of Atheroma[62-65]:

The endothelial cells which are attached to the blood in the lumen of the arteries are rested on the basement membrane. The elastic lamina situated internally forms the barrier between the tunica intima and media. The tunica media contains multiple layers of SM cells, packed tightly than in intima, embedded in a matrix which has abundant elastin and collagen. In early atherogenesis, inflammatory cell recruitment and lipid accumulation lead to development of lipid rich core leading to arterial enlargement. If inflammation and other risk factors persist, the lipid core grows, and proteinases secreted by the activated leukocytes degrade the ECM, while pro-inflammatory cytokines like IFNγ limit the production of new collagen. Above changes thin the fibrous cap and make it friable and liable to rupture. After rupture, blood coming in contact with the tissue factor in the plaque coagulates. Activation of platelets by thrombin generated from the coagulation cascade and by contact with the intimal compartment initiates the thrombus formation. If the thrombus is occluding the blood vessel continuously, it results in an acute myocardial infarction. A wound healing response which is triggered by thrombin generated during blood coagulation stimulates smooth muscle proliferation. PDGF released from activated platelets stimulates smooth muscle cell migration. TFG-b, also released from activated platelets, stimulates interstitial collagen production. This increased migration, proliferation and extracellular matrix synthesis by smooth muscle cells thickens the fibrous cap and causes further expansion of the intima, often now

in an inward direction, yielding constriction of the lumen. Stenotic lesions produced by the fibrosed plaque due to encroachment into the lumen will restrict the flow, especially during increased cardiac demand, leading to ischaemia, commonly provoking symptoms such as angina pectoris. Advanced stenotic plaques, which are more fibrous are less susceptible to rupture and renewed thrombosis. Lipid lowering can decrease the content of lipid and slow down the intimal inflammatory response, which in turn provide a more ‘stable’ plaque with a thick fibrous cap and a preserved lumen.

Figure 3.9 Evaluation of atherosclerosis[66]



Hypothyroidism and atherosclerotic risk factors[67]:

Observational studies done previously have proven the idea that hypothyroidism accelerate atherosclerosis. A study showed that severe atherosclerosis was found in 84% myxedematous patients[68, 69]. Although controversy still exists, a meta-analysis showed that subclinical hypothyroidism is significantly associated with coronary heart disease at baseline and death from cardiovascular causes at follow-up[70]. However, it remains to be determined whether thyroxine replacement therapy reduces the risk of coronary heart disease in these patients. A study on the progression of coronary atherosclerosis in patients undergoing coronary angiography showed that treatment of hypothyroidism affected atherogenesis, although the number of patients enrolled was very small[71]. Nagasaki et al. showed that IMT inpatients with overt hypothyroidism was significantly higher compared with that in euthyroid control subject and 1-year treatment with levothyroxine reversed the increased IMT in hypothyroid[72].

Multiple studies clinically have proven that lot of factors are responsible and related to hypothyroidism like lipid abnormalities, dysfunction of endothelium, inflammation present chronically, insulin resistance and oxidative stress. Those studies also revealed these factors as a whole is not responsible and they interact between them and no factor is as effective alone

Hypercholesterolemia has been found to be one of the factors contributing for atherosclerosis. LDL is more susceptible to oxidation process which increases the atherogenicity in hypothyroid patients[73]. In addition HDL

concentration which is inversely related to atherosclerosis, was found to be decreased in hypothyroidism[74]. Therefore, the lipid profile in patients with overt hypothyroidism is generally prone to enhancement of atherosclerosis.

Chronic inflammation is one of the factors which induce and stimulate atherosclerosis and related complications by having deleterious effects endothelium of blood vessels, this may be the reason due to which in hypothyroidism patients there may be increase in dysfunction of endothelium. Many mediators of inflammation like IL-6, TNF-α and CRP, are related to hypothyroidism. Interleukin - 6, is induced in preadipocytes by TSH which has harmful effects like atherosclerosis and damage to endothelium it is pro inflammatory cytokine. Another similar cytokine related with inflammation TNF-α has effect on NO activity. It damages activity of NO in endothelial cells .TNF alpha promotes oxidative stress which is a main facet of malfunction of endothelium, this is induced by TSH in bone marrow cells. Later it was proven that liver generated C reactive protein which is an acute phase protein is also linked with inflammation. It's was later identified to be an important marker which is having part in inflammation ending up in cardiovascular events. This CRP has a positive relation with TSH and in hypothyroidism patients appears to be elevated[86, 87].

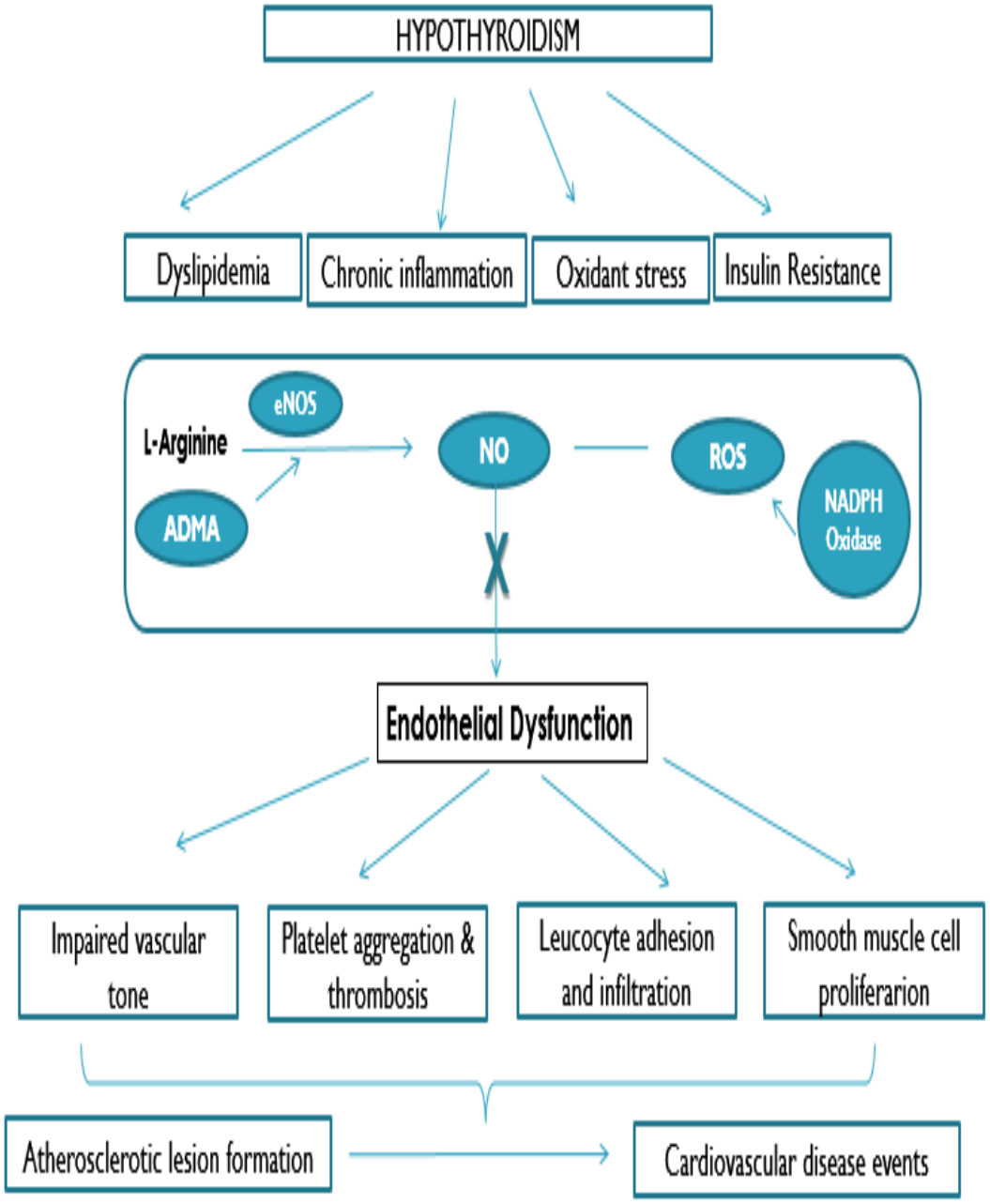
Oxidant stress is an imbalance between oxidant and antioxidant substances, ROS, a family of molecules including hydroxyl radical, superoxide anion and their derivatives, exceed endogenous antioxidant defence mechanisms. Inflammation is one of the important causes of

oxidative stress as enzymatic systems produce a large amount of ROS, including xanthine oxidase and NADH/NADH phosphate oxidase induced by inflammatory stimuli. Since ROS reacts with NO readily, producing harmful reactive nitric intermediates, minimum oxidative stress in endothelial cells can uncouple NO synthesis and will be devastating to endothelial function. Induction of iNOS can therefore aggravate oxidative stress in inflammatory conditions. ROS can also impair endothelial function by activating NF-κB, which further increases the expression of inflammation-associated genes, and thus act as a negative feedback loop[88, 89].

Insulin resistance is associated with dyslipidemia, chronic inflammation and oxidative stress, which are aspects of metabolic syndrome. As mentioned above other three factors directly promote endothelial dysfunction, whereas insulin resistance is usually linked with metabolic syndrome with a minor direct action on endothelium. Metabolic syndrome has been taken as an important underlying cause for the majority of the cardiovascular diseases[90].

Endothelial dysfunction is generally accepted as an early step of the atherosclerosis. Measurement of flow-mediated endothelium-dependent vasodilatation[75] indicated an association between hypothyroidism and atherosclerosis. A reduction in the availability of nitric oxide is suggested as the mechanism of endothelial dysfunction in hypothyroidism[76]. Few studies have proven that thyroid hormone replacement improved endothelial function in patients with hypothyroidism[77].

Figure 3.10 Subclinical hypothyroidism induced endothelial dysfunction[78]



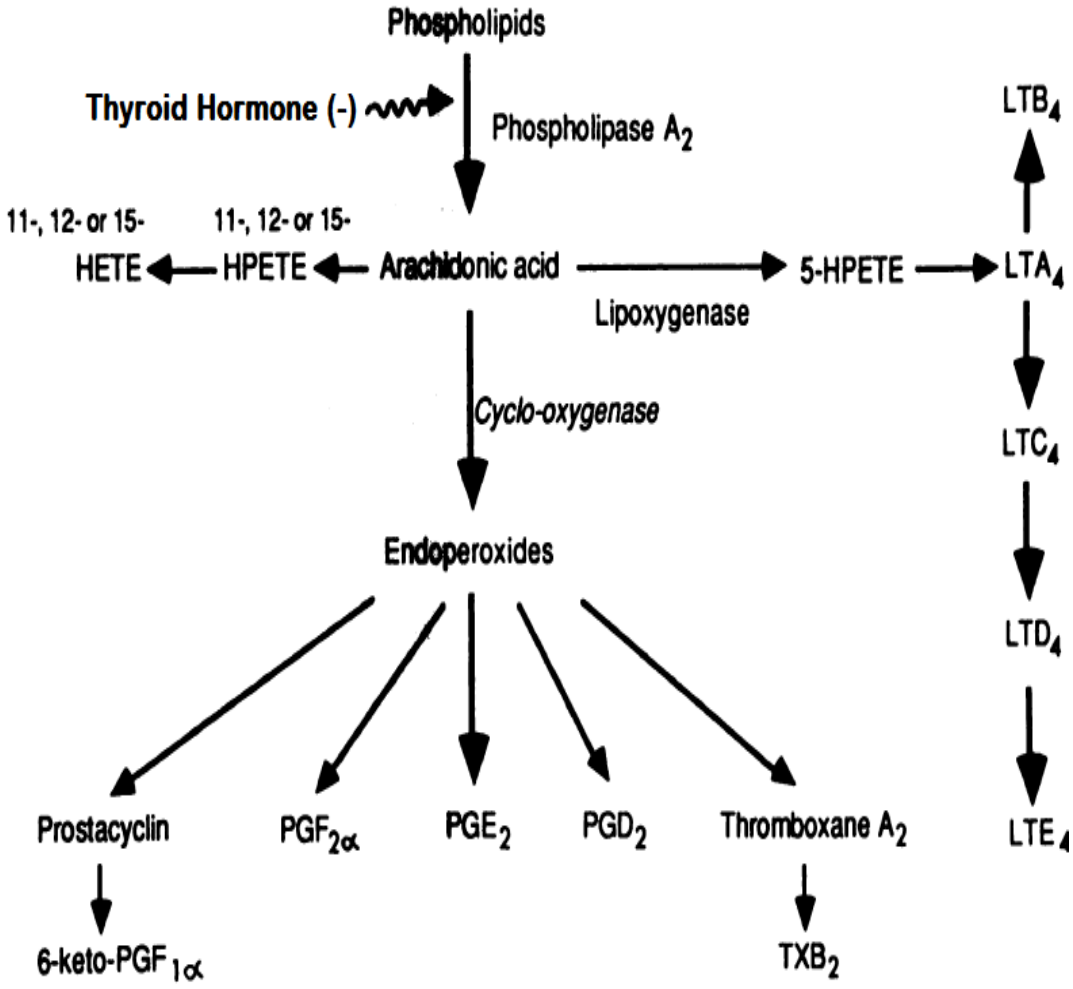
Hormones secreted by thyroid have various effects on vascular system and heart. Thyroid hormone (TH) exerts multiple effects on the heart and vascular system. Obvious hypothyroidism when present in association with hypertension and dyslipidaemia mostly ends up in CVD. Also if the patient is having subclinical hypothyroidism which presents with high TSH and normal T3 is an important indicator of MI and atherosclerosis. So it will be better to focus on this factor to find the interaction or relation between TSH and CVD[79,80].

Phospholipase A2 group 2A (PLA2G2A):

Phospholipase A2 (PLA2) is an esterase that hydrolyzes membrane phospholipids at the sn2 position to generate free fatty acids like arachidonic acid and lysophospholipids. Arachidonic acid serves as a precursor for the synthesis of prostaglandins and leukotrienes. PLA2s are classified into three main families including the Ca2+ dependent secretory PLA2s, the cytosolic PLA2s, and the Ca2 + independent phospholipases. The phospholipaseA2 group IIa (PLA2G2A) isoform belongs to the family of secretory PLA2 (sPLA2). Elevated levels of PLA2G2A are observed in many diseases associated with inflammation including rheumatoid arthritis, pancreatitis, and septic shock. PLA2G2A contributes to the development of atherosclerosis. It not only acts on membrane phospholipids but also targets lipoproteins and dietary phospholipids. Consistent with its role in inflammation, PLA2G2A is expressed in macrophages, but it is also highly expressed in hepatocytes. Expression of the PLA2G2A gene is induced by cytokines including TNF α,

and IL-6. At the transcriptional level, PLA2G2A is stimulated by the nuclear factors NF-KB[81, 82].

Figure 3.11 Relation of Thyroid hormone and PLA2G2A[84]



A study was done in Tennessee, USA which showed that Thyroid hormone (T3) inhibits PLA2G2A transcription via binding of the T3 receptor (TR beta) and signifies that T3 regulates PLA2G2A expression and utilizes a novel mechanism of co repressor recruitment. Another study was done in Iran

in subclinical hypothyroid (SCH) patients to explore the possible association between SCH and the inflammatory marker PLA2G2A which showed a stronger association of SCH with PLA2G2A indicating that PLA2G2A is an inducer of inflammation[83]. There are very studies available regarding the association of PLA2G2A in development of atherosclerosis in hypothyroidism[84].

Adiponectin (ADP):

ADP is a protein containing 244 AA constituting most copious gene of the adipose tissue, gene transcript 1. Almost 0.01% of total plasma proteins is occupied by ADP and range of plasma concentrations is five to thirty mg/ml[85].

There are 2 types of receptors for ADP (AdipoR), So far 1 and 2 is well-known. Primary site of expression of AdipoR1 is muscle and that of AdipoR2 is liver. ADP binds to AdipoR1 and AdipoR2 thereby causing increased glucose uptake followed by fatty acid oxidation in the skeletal muscle through AdipoR1, as a result reduced glucose output from the liver through AdipoR2[91].

There are 3 forms of circulation ADP

- Trimer
- Hexamer
- HMW multimers

The physiological effects of ADP are mainly related to concentration in plasma, ADP isoforms with its related properties subtypes and its tissue-specific expression. The most active form of ADP is the HMW[92]. They have different properties like

- Anti-atherogenic
- Anti-diabetic, and
- Anti-inflammatory properties.

Many diseases are related to decreased levels of ADP such as

1. Atherosclerosis
2. Type 2 diabetes
3. Low HDL
4. Abdominal obesity
5. Hypertension,
6. Metabolic syndrome
7. Hypertriglyceridemia

Some biological actions of ADP acts together with thyroid hormones like reduction of body fat by increased thermogenesis and lipid oxidation, it is plausible that ADP may interrelate with thyroid axis[92]. These hormones are linked with insulin resistance, and association between ADP and thyroid hormones may present through direct or indirect communications between them. These hormones are prime controllers of metabolism of lipid and carbohydrate. Dysfunction of thyroid sometimes ends up in dyslipidaemia and diminishing of glucose metabolism. The reasons behind these abnormalities

are still unknown. The receptors were revealed to have important part in lipid and glucose metabolism. Abnormalities in these receptors causes rise in TGL content in tissue, leading to increased oxidative stress and inflammation which ends up in prominent glucose intolerance and insulin resistance. As the incidence of hypothyroidism increases with the age and body weight inverse relation in ADP level found in few studies.[97, 98]

Many studies in animal with hypo or hyperthyroid state revealed debatable results about relation of thyroid hormones with ADP levels. Rats with hypothyroidism had different results with normal or increased levels of serum ADP. Another study revealed that response of ADP mRNA to changes in T3 levels depends on type and site of the White adipose tissue. Contrary to that, there was rise in ADP mRNA expression and release, while it was vice versa with methimazole in hypothyroid rats resulting in reduction in expression of ADP mRNA[93].

Among few studies done in humans there were contradictory results on ADP and thyroid hormone interaction. A research was done in Turkey they analysed levels of ADP levels in women clinically identified as subclinical hypothyroidism also possible actions L-thyroxine on those levels. The study revealed that after L-thyroxine replacement for six months there was improvement in ADP levels which was not dependent on body fat mass[94].

Only very few studies were available on hypothyroidism and chronic inflammation and they were done in other parts of the world. Very few human studies were done on hypothyroidism and inflammatory markers PLA2G2A

and ADP. But they were also done as separate studies. Also no studies were found regarding the relationship between doses of thyroxine and its effect on inflammation. Though few similar studies have already done in other countries, ethnic variations, life style modifications and food habits differ in our country comparing to them. So we proposed this study to compare the status of inflammation in hypothyroidism with these inflammatory markers and also to explore the possible relationship between drug treatment and the risk of atherosclerosis[95, 96].

Methodology

METHODOLOGY:

STUDY AREA:

PSG Hospitals, Coimbatore

STUDY DESIGN:

This study was a clinical prospective cross sectional study. After obtaining informed consent from the patients fulfilling the inclusion and exclusion criteria, samples of blood were collected, stored and data collection done during the same visit.

STUDY POPULATION:

Outpatients of General Medicine & Endocrinology Departments of PSG Hospitals with clinical diagnosis of hypothyroidism.

SAMPLE SIZE:

Prevalence of hypothyroidism around 4%, in South Indian Study (1) hence sample size based on formula $4\ p\ q/d^2$

$= 4 \times 4 \times 96/ 25 = 61$

Rounding off to 60 patients

INCLUSION CRITERIA:

Patients attending medicine OPD of age >18years with clinical diagnosis of hypothyroidism including old and new patients.

EXCLUSION CRITERIA:

1. Post operated and Radiation cases of thyroid are excluded
2. Patients on Statins, Steroids, Metformin are excluded
3. Age<18 years are excluded
4. Those who on other system medication (ayurveda, siddha)

ETHICAL APPROVAL:

The proposal of study was accepted by institutional Human Ethics Committee (IHEC) preceding start of the study. The details and the purpose of the study protocol were explained to each participant individually and their doubts were clarified before obtaining informed consent. The informed consent forms were provided to the participants either in English or Tamil depending on the participant. The participants, who gave written informed consent and eligible for recruitment according to the inclusion criteria, were enrolled for the study. A copy of the consent form is attached in the annexure.

Tool used:

- Data collection form
- ELISA kit for analysis of PLA2G2A (CUSABIO PLA2G2A ELISA kit) and Adiponectin (RAY BIO Human Acrp30 ELISA kit)

Sample collection and storage:

Samples collected and separated using serum separator tube after allowing clotting for two hours at room temperature. Centrifugation done for 15 minutes 1000 RPM then stored at -20°C**.**

Analysis done:

ECLIA test for TSH - from Biochemistry lab

ELISA for PLA2G2A and Adiponectin - done

Human Phospholipase A2 group 2A (PLA2G2A):

PLA2G2A was estimated using CUSABIO PLA2G2A ELISA kit imported from USA.

Methodology is through EIA to specify quantitatively estimated. A microplate with precoated Phospholipase A2 is used .Wells were kept and test and standard solution were added to those wells and PLA2 if present bind with the antibody (Ab). Then biotin layered Ab with high specificity for PLA2 is added, then avidin conjugated HRP was assay followed by substrate buffer. When colour develops the intensity is measured.

Level of Measurement (Range):

1.56ng-100 ng per millilitres

Storage:

The ELISA kit was unopened and kept at 2-8 °C

How reagent is prepared:

After bringing the reagents to room temperature, freshly prepared standards are used. These standards should be used with in 4 hrs of preparation. Always distilled water should be used.

Figure 4.1 Reagent Preparation

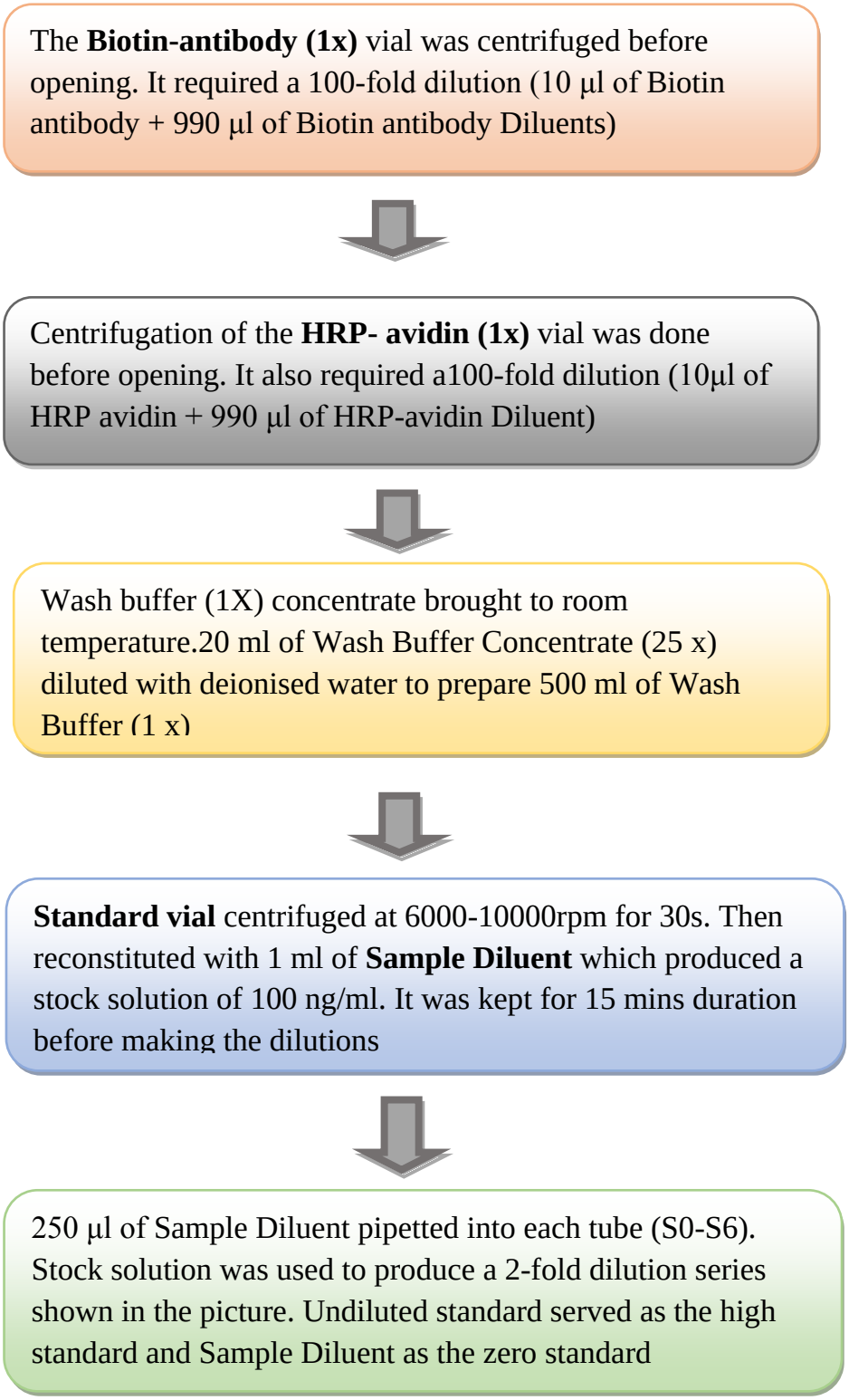
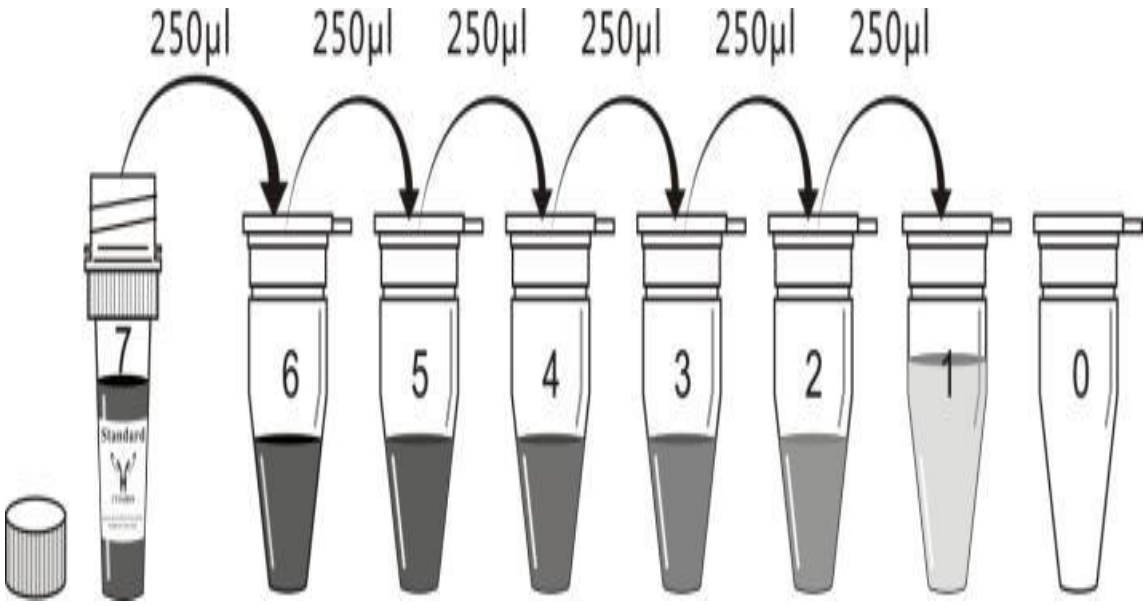
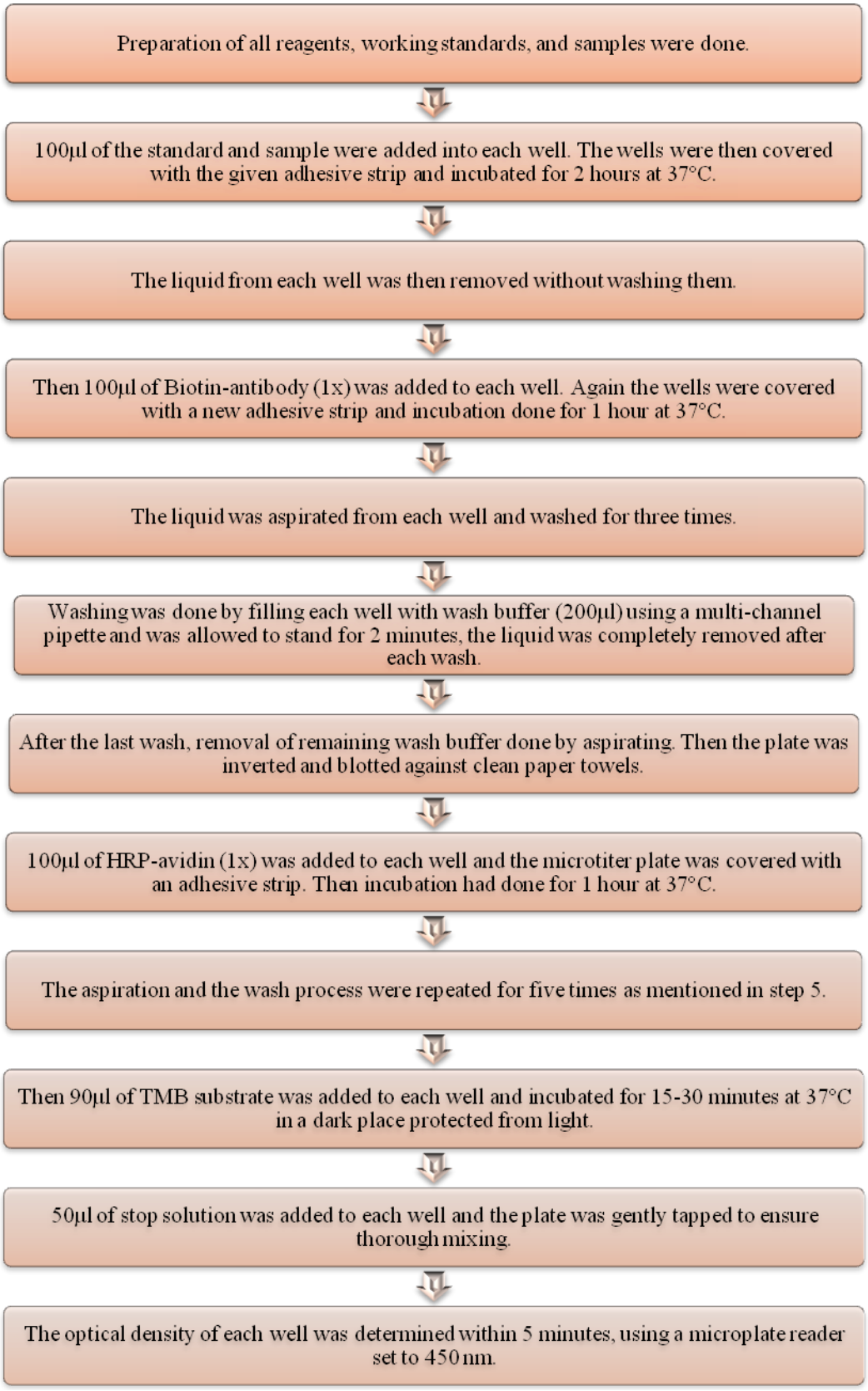


Figure 4.2 Dilution series of sample



Tube	S7	S6	S5	S4	S3	S2	S1	S0
ng/ml	100	50	25	12.5	6.25	3.12	1.56	0

Assay procedure:



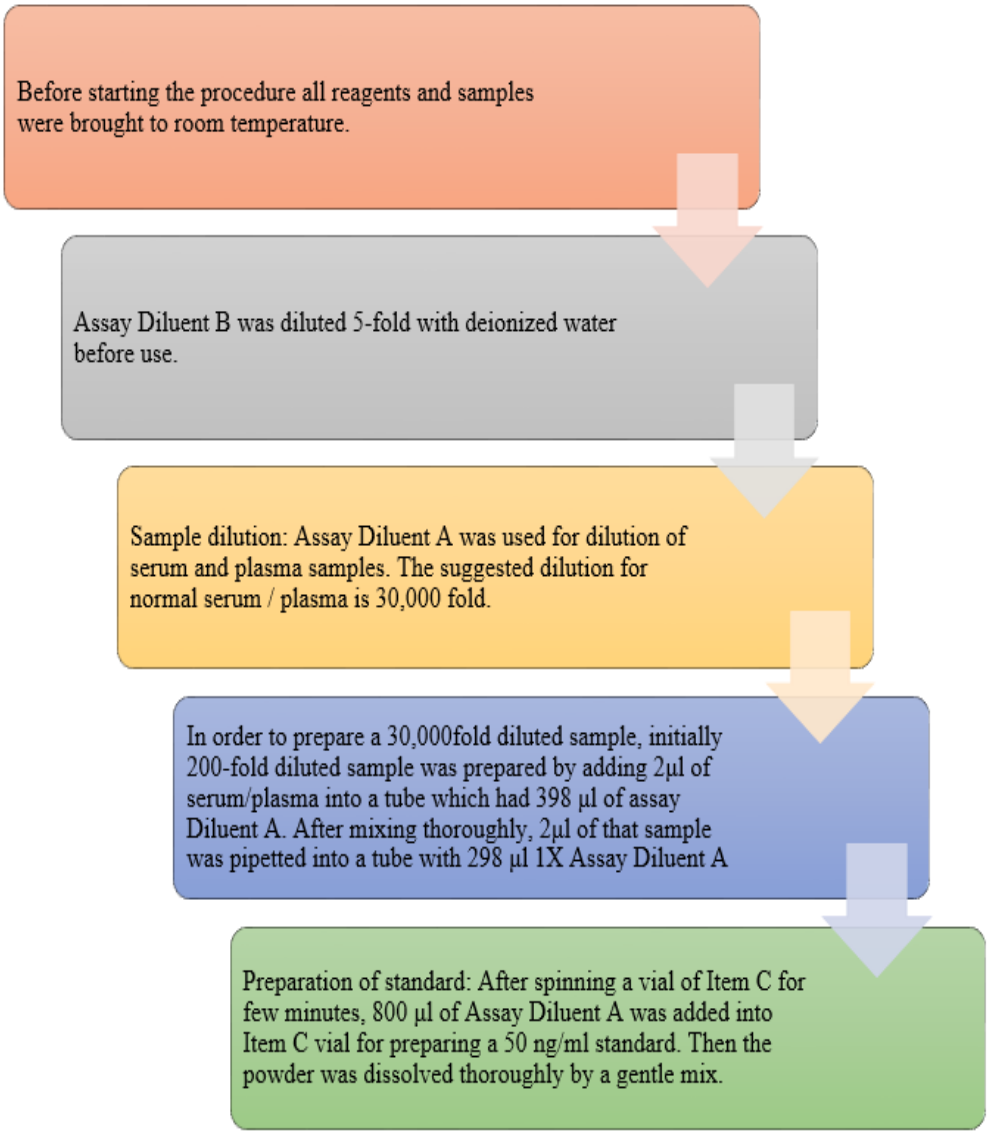
Adiponectin:

The Adiponectin was estimated using a ELISA kit named **Ray Bio Human Acrp30 ELISA Kit**, imported from Georgia, USA.

Storage:

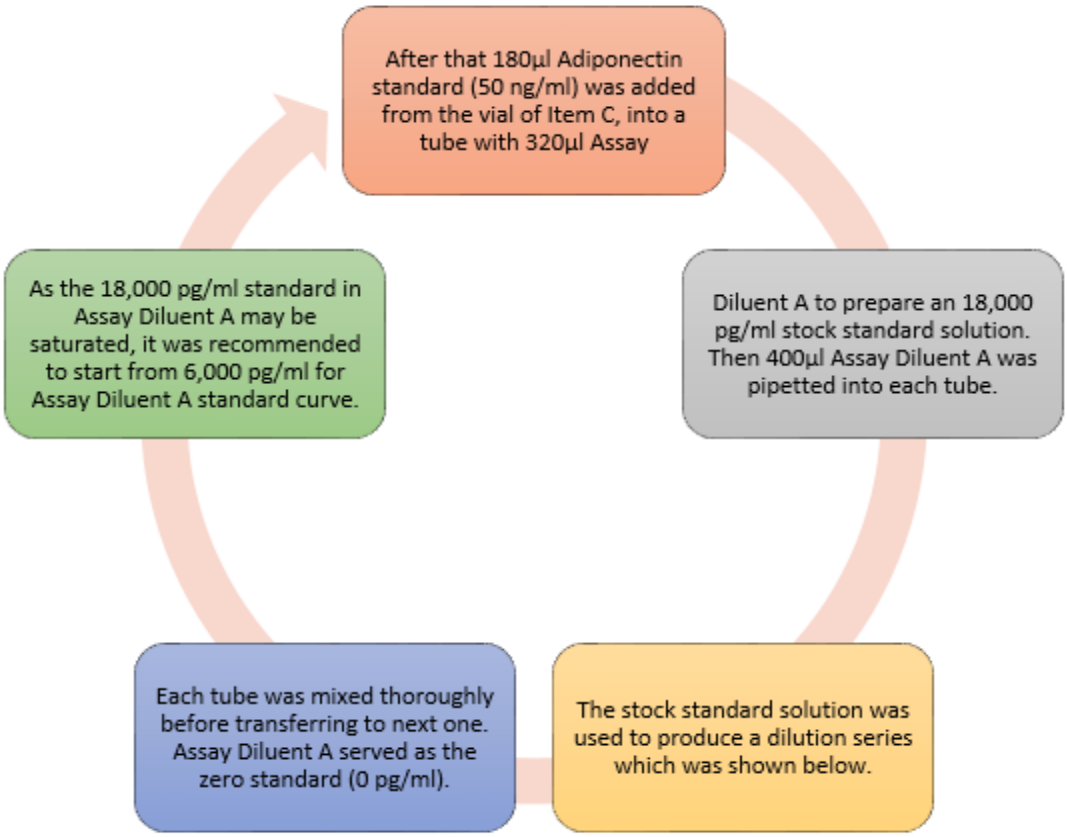
Stored in deep freezer at -20ºC

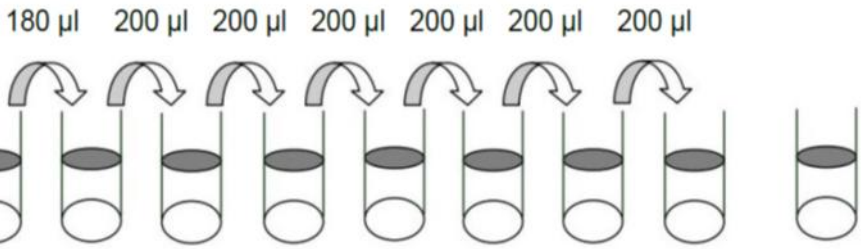
Figure 4.3 Preparation of Reagent:



Followed by:

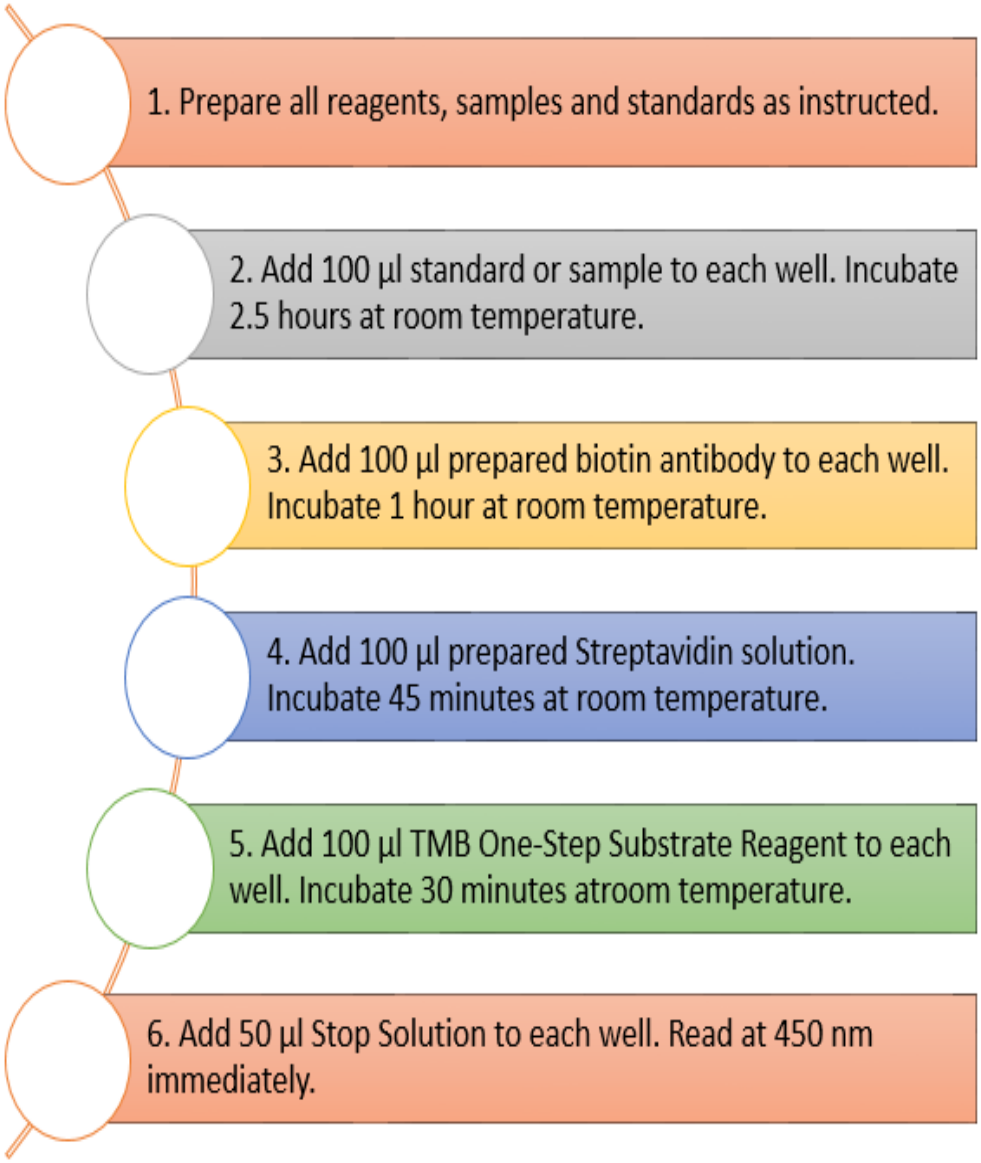
Figure 4.4 Serial Dilutions





		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 800 µl	320 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	50 ng/ml	18000 pg/ml	6000 pg/ml	2000 pg/ml	666.7 pg/ml	222.2 pg/ml	74.07 pg/ml	24.69 pg/ml		0 pg/ml

Assay procedure:

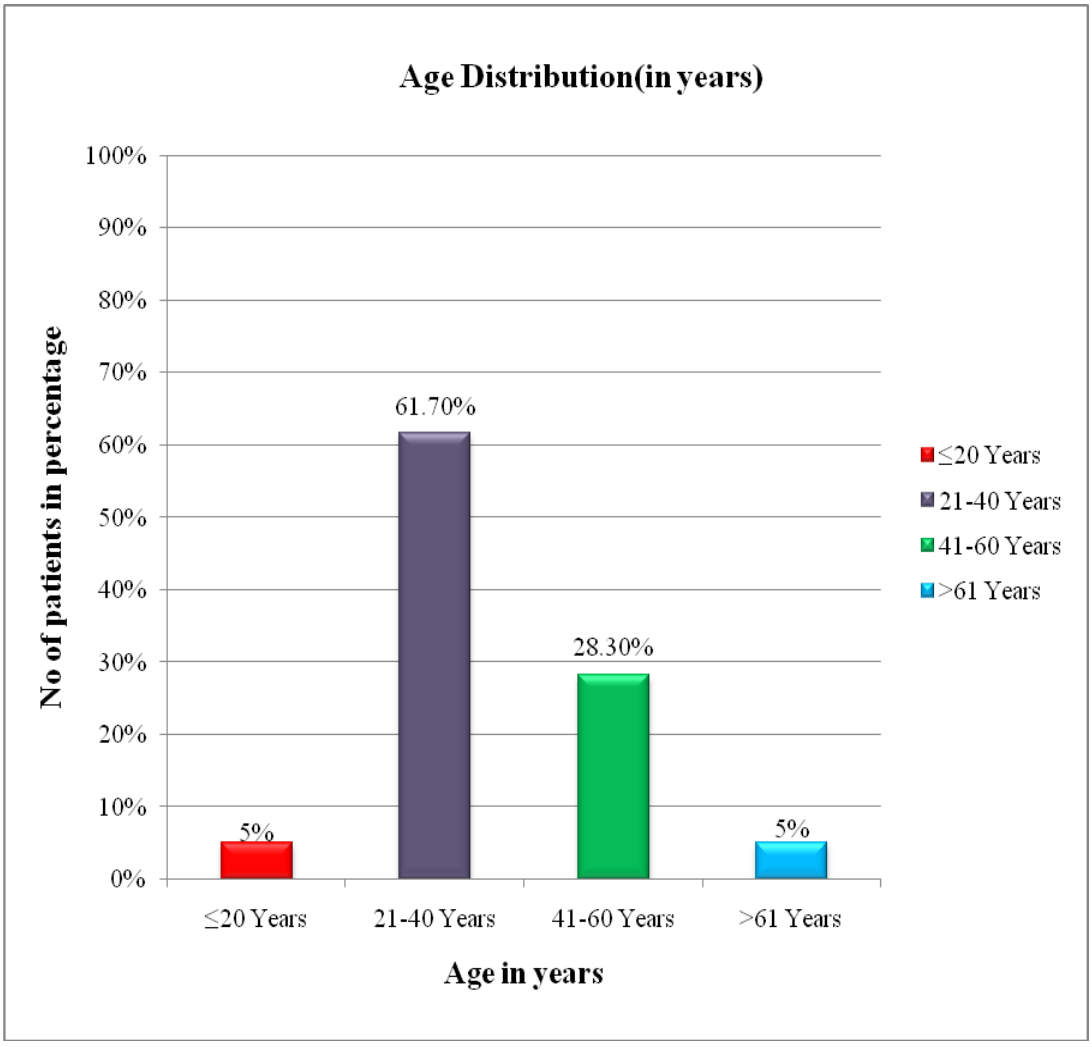


The plates were then inserted into an ELISA reader and the absorbance read. ELISA reader is a Bio-Rad system which is attached to a computer.

Results

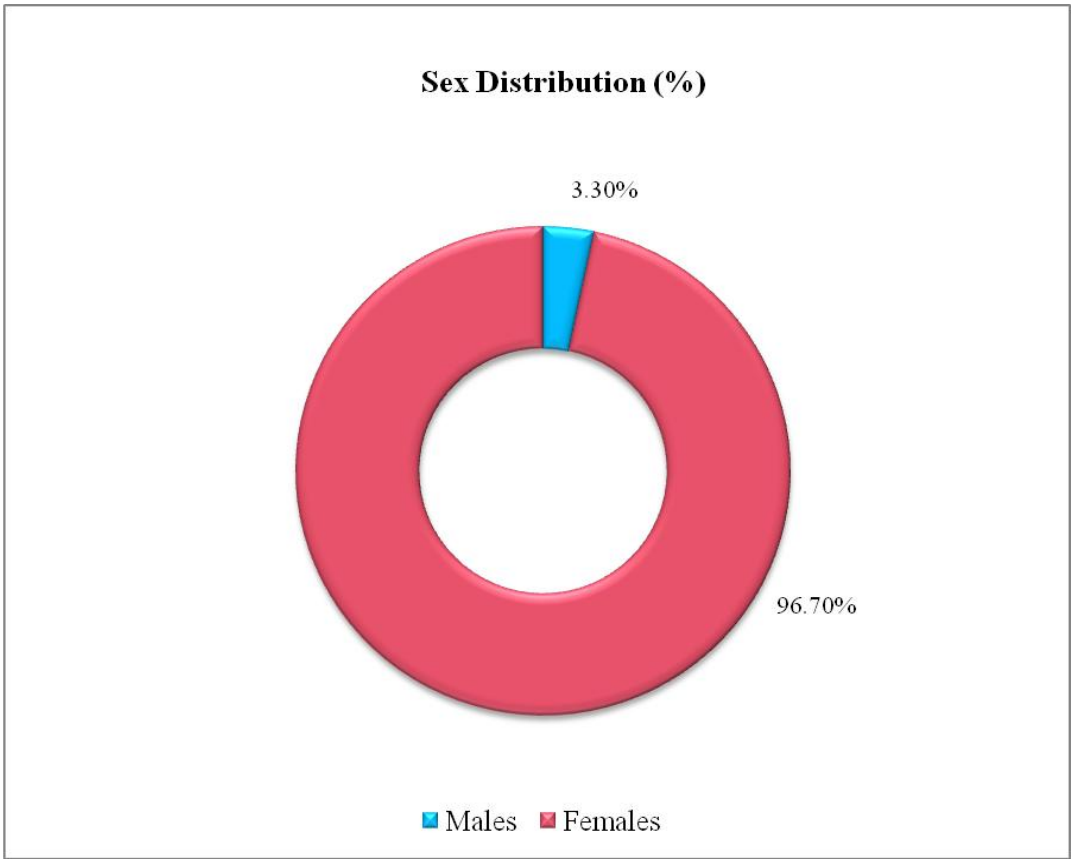
RESULTS:

Figure1. Age Distribution (in years)



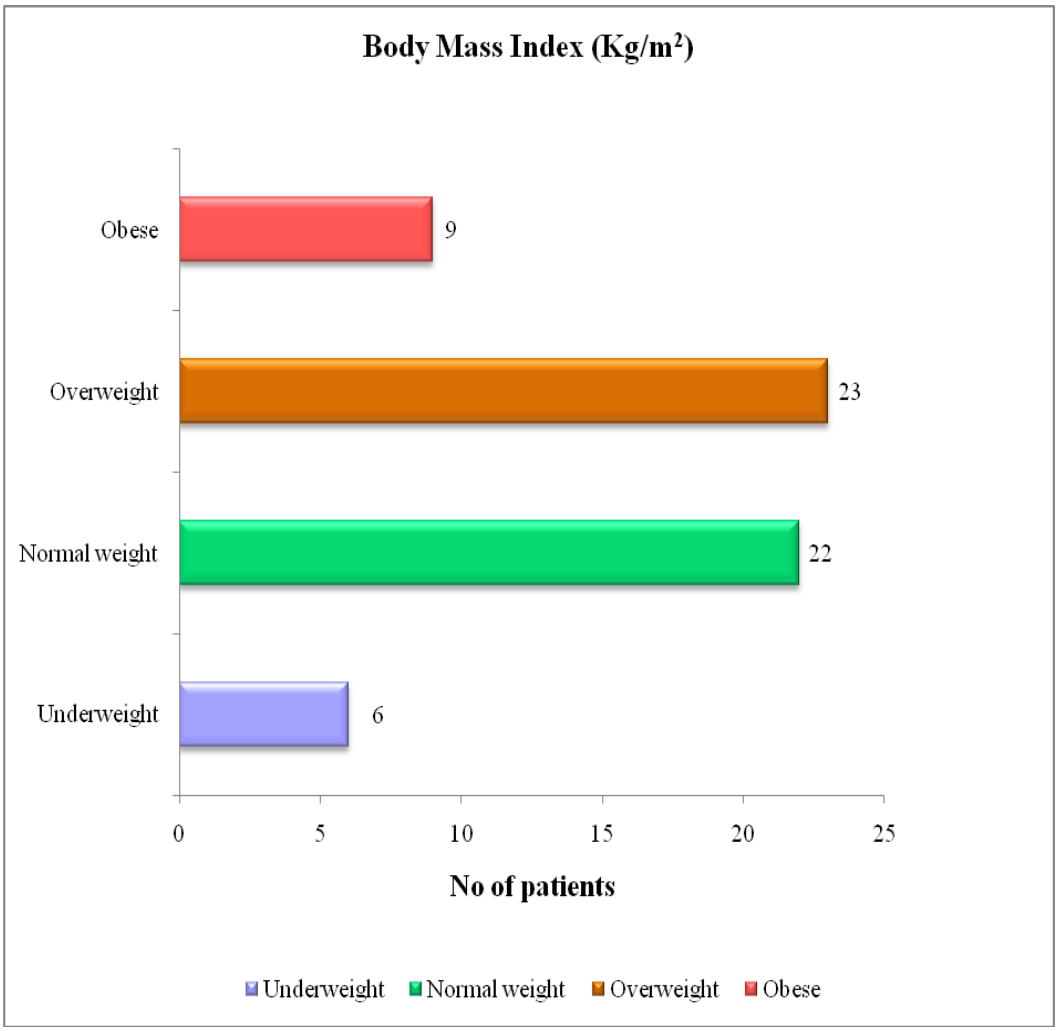
Among the 60 patients included in our study, most of them 61.7%(37) were from 21 to 40 years of age, 28.3% (17) were from 41 to 60 years, with only 5% (3)patients in both ≤ 20 years and > 61year age groups.

Figure 2. Sex Distribution (%)



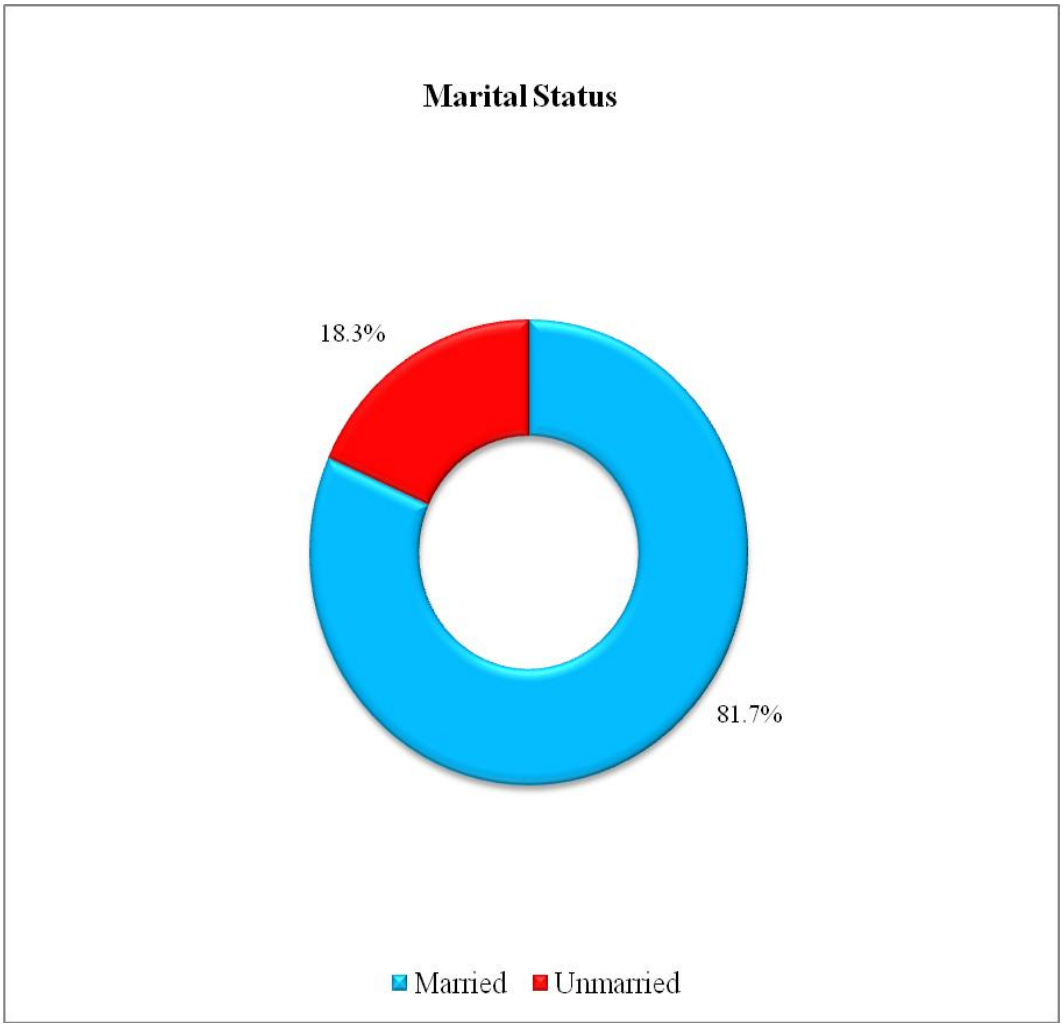
Among 60 patients 58 (96.7%) were female patients with only 2 (3.3%) were male patients.

Figure 3. Body mass index (BMI)



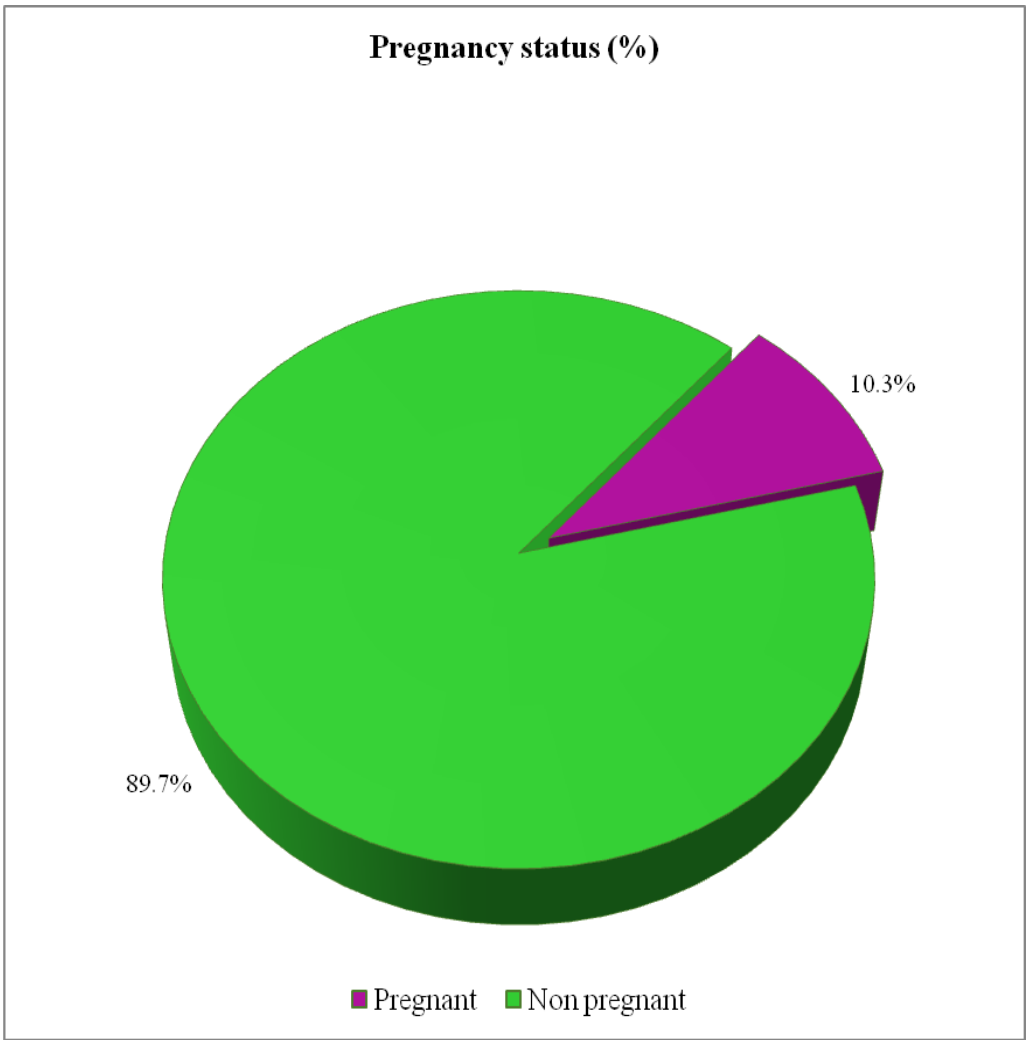
In our study 23 (38.3%) patients were overweight, while 22(36.7%) were of normal weight and 9 (15%) patients were obese with only 6 (10%) patients been underweight.

Figure 4. Marital status



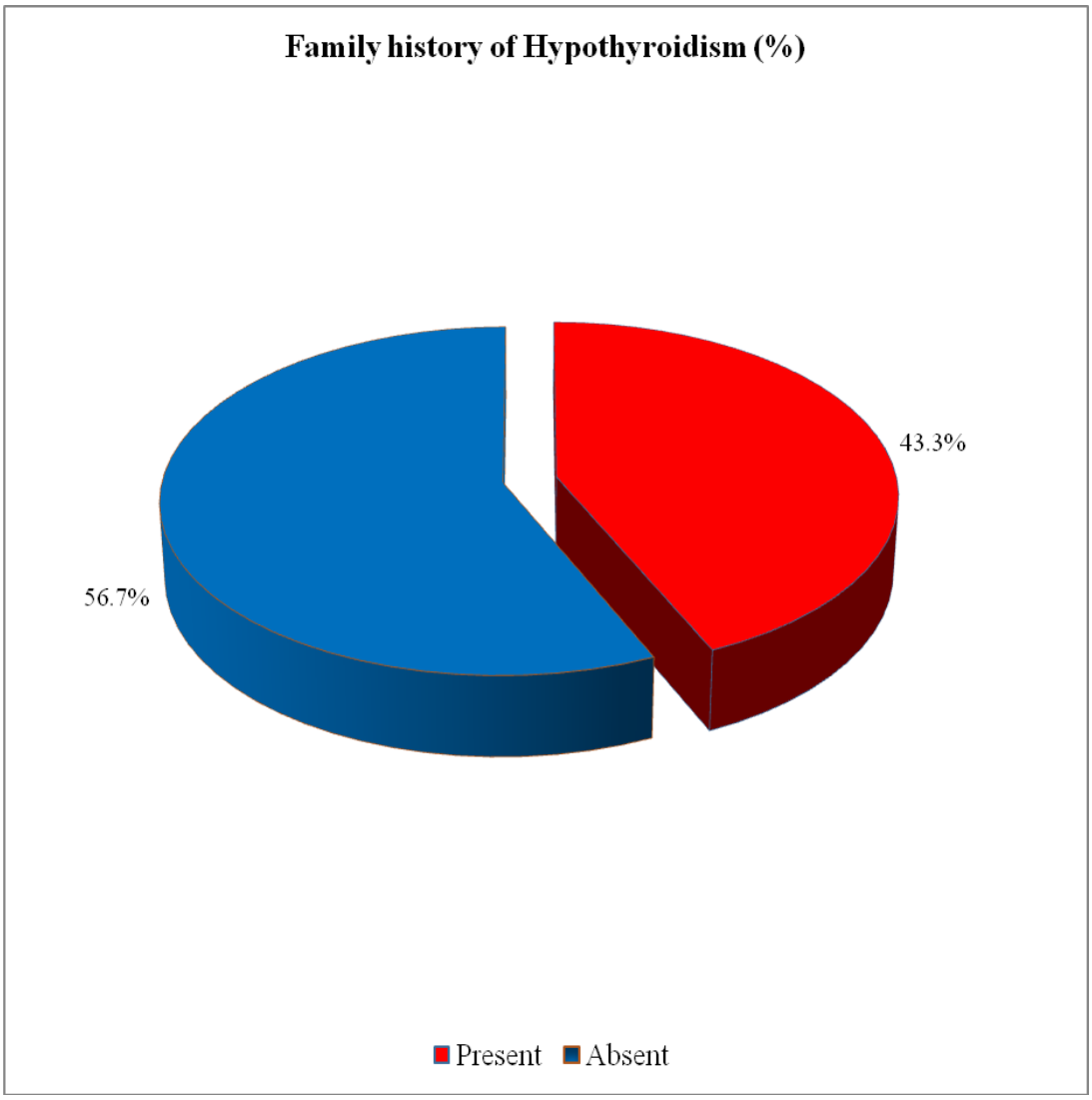
Among the 60 hypothyroid patients included in the study, 49(81.7%) patients were married and the remaining 11(18.3%) were unmarried.

Figure 5.Pregnancy status



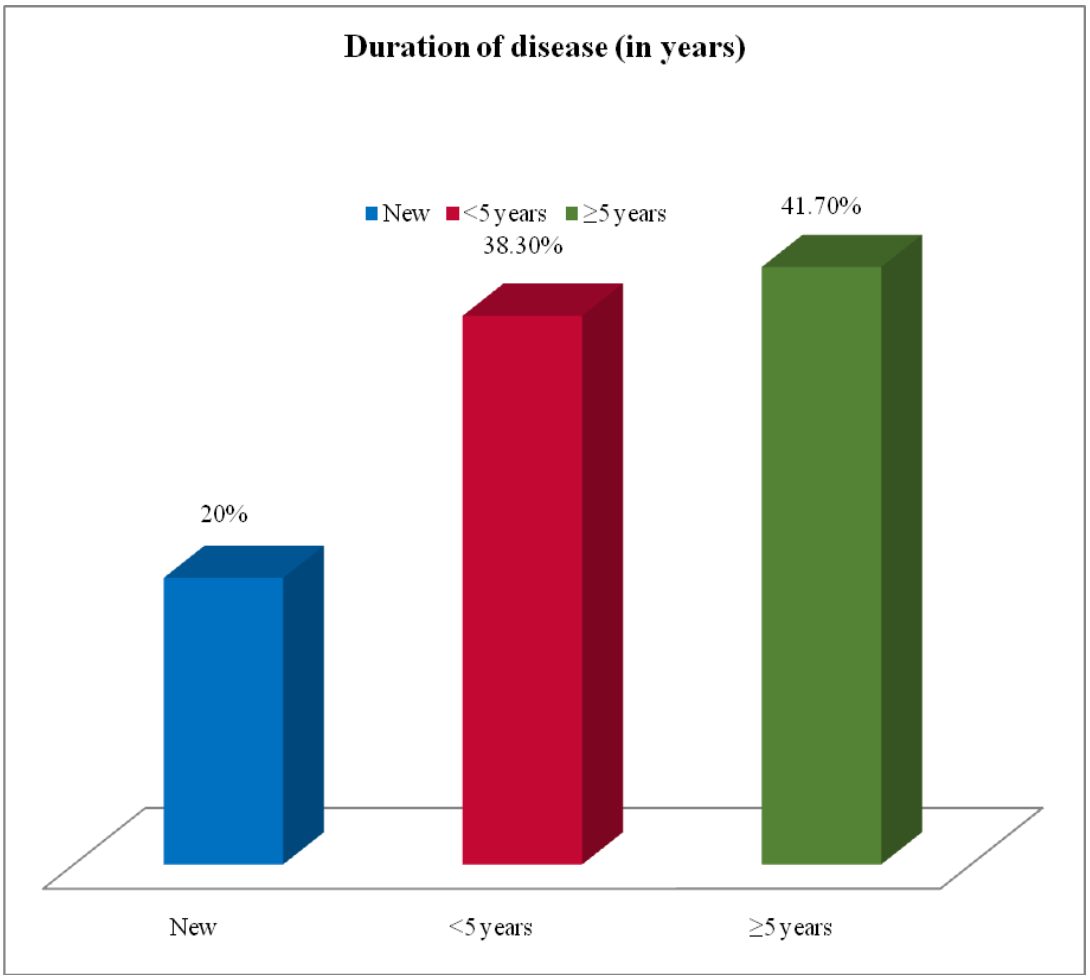
In our study group, among those 58 female patients recruited, 6 (10.3%) patients were pregnant during the study period.

Figure 6.Family history



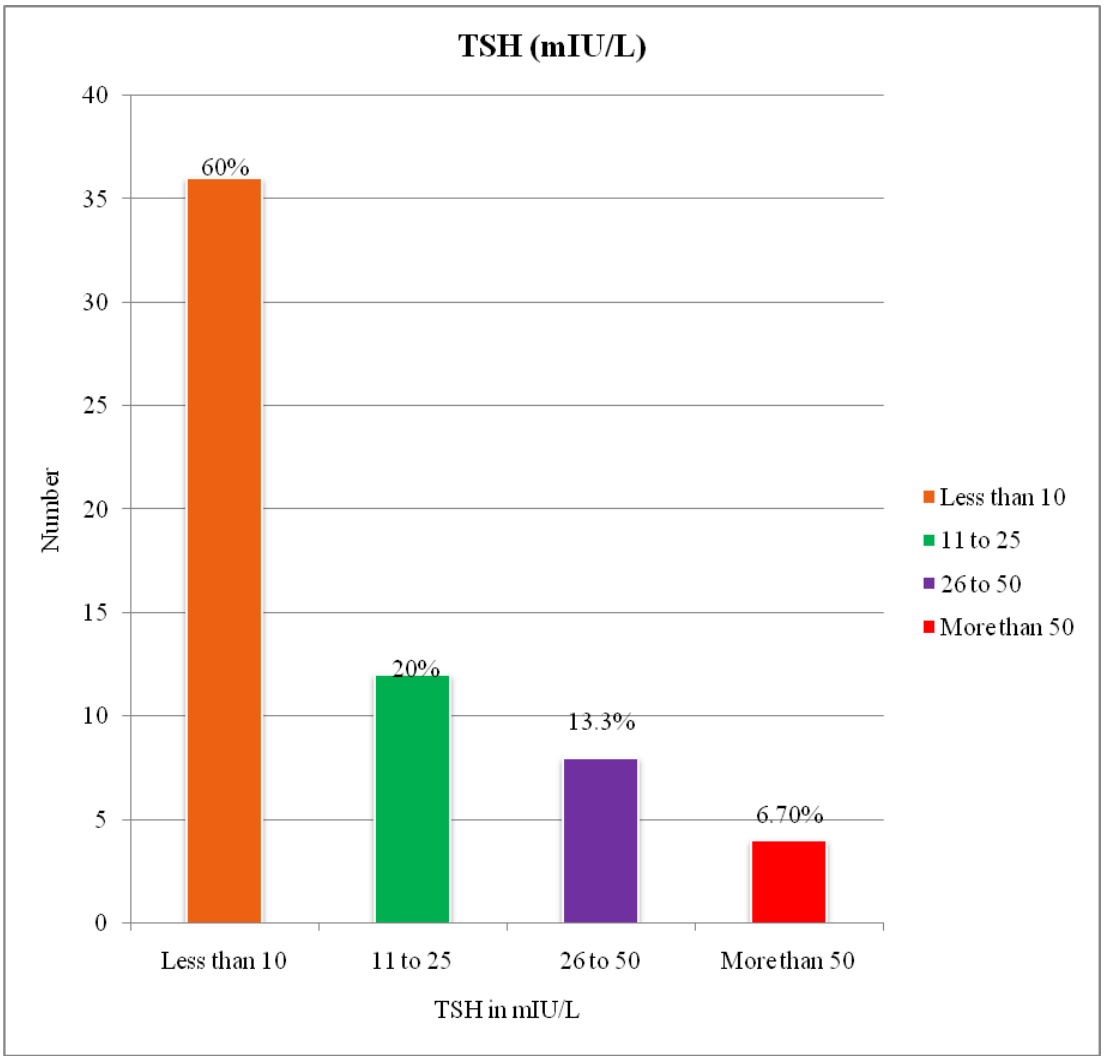
Among the subjects included in our study, 26(43.3%) had a positive family history of hypothyroidism and the remaining 34(56.7%) patients had no other significant family history.

Figure 7.Duration of disease



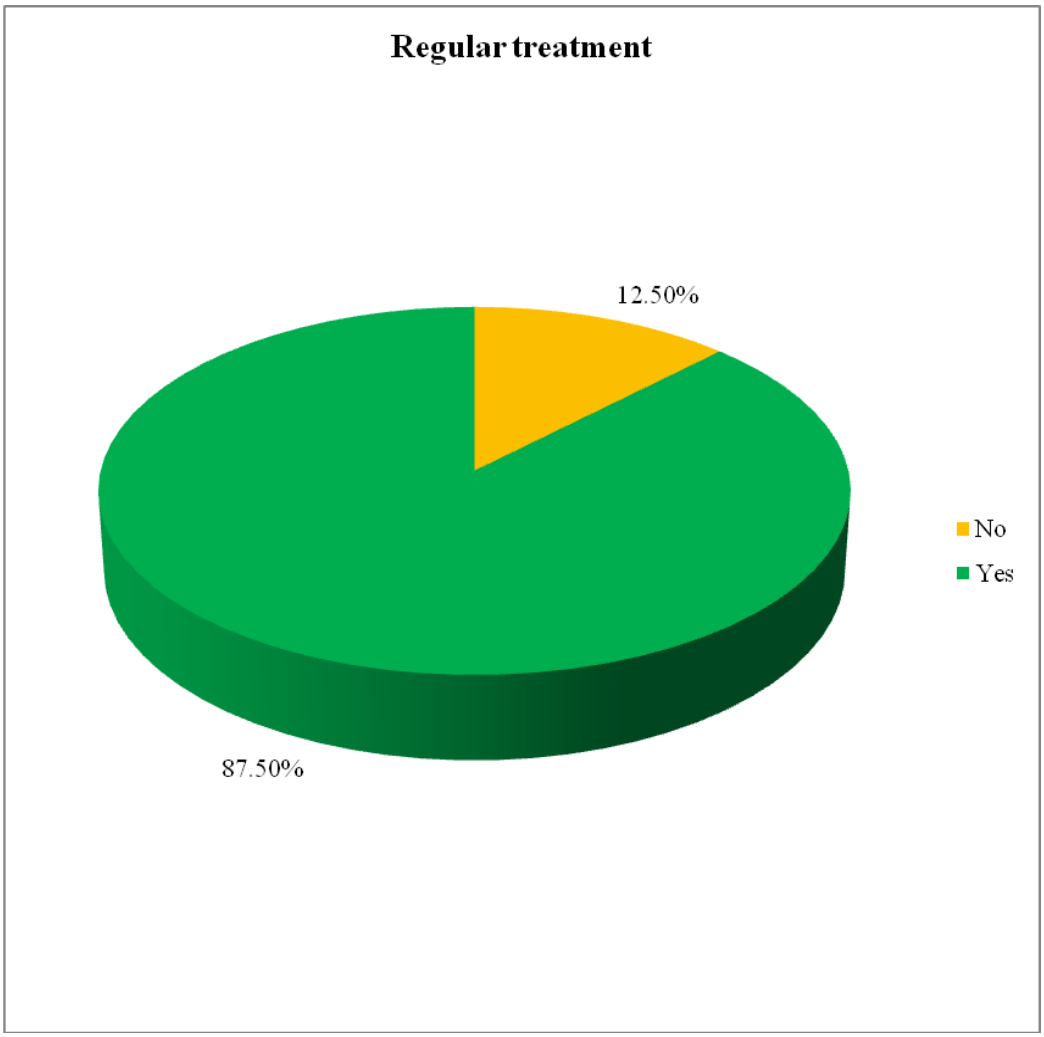
In our study group both the duration of the disease and the duration of treatment for hypothyroidism were found to be the same. 12 (20%) were newly diagnosed hypothyroid patients, 23(38.3%) were hypothyroid for less than 5 years while 25 (41.7%) were diagnosed with the disease since 5 years or more.

Figure 8.TSH values (mIU/L)



Among the 60 hypothyroid patients, 36 (60%) had TSH value of less than 10, 12(20%) had between 11 to 25, with 8(13.3%) patients had between 26 to 50, while only 4(6.7%) of them had more than 50.

Figure 9.Regular treatment

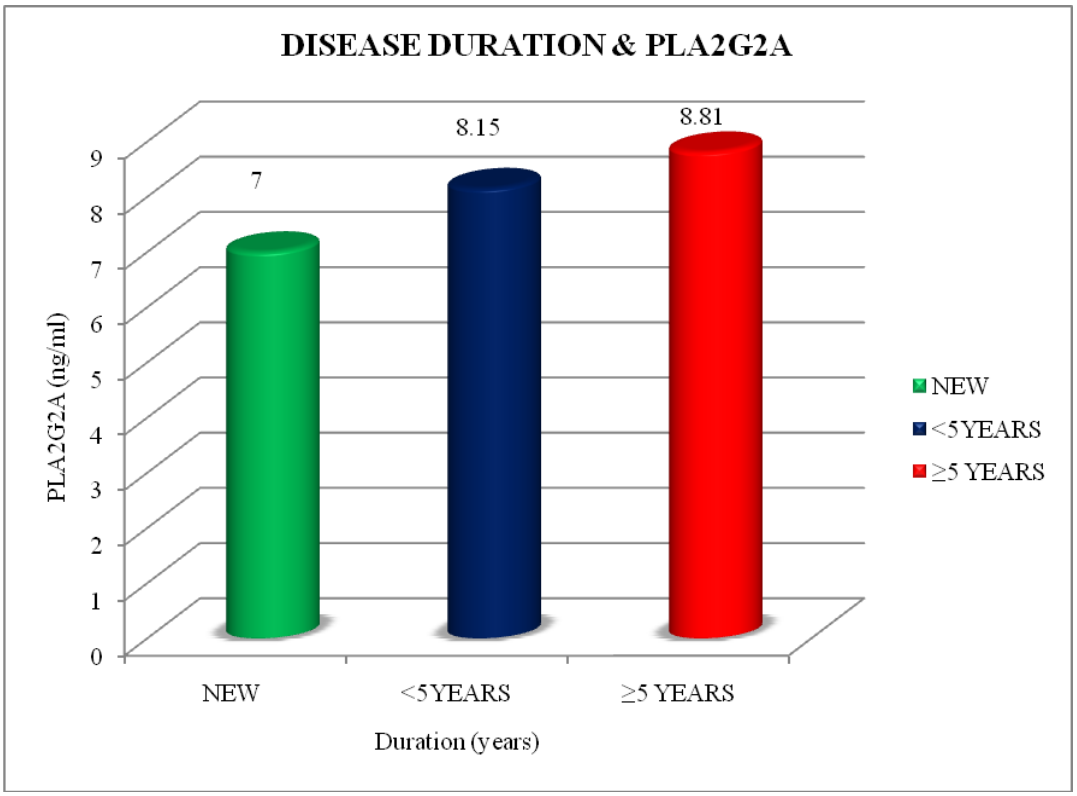


Looking at the compliance of drug intake by the patients, among those 48 hypothyroid patients who were on thyroxine treatment excluding the new onset patients, 42 (87.5%) were on regular treatment while the remaining 6 (12.5%) were not on regular treatment.

Table 10.Disease duration & PLA2G2A

Duration	Number	Mean	SD
0years(New)	12	7.00	4.15
<5 years	23	8.15	4.54
≥5years	25	8.81	3.68

Figure 10.Disease duration & PLA2G2A

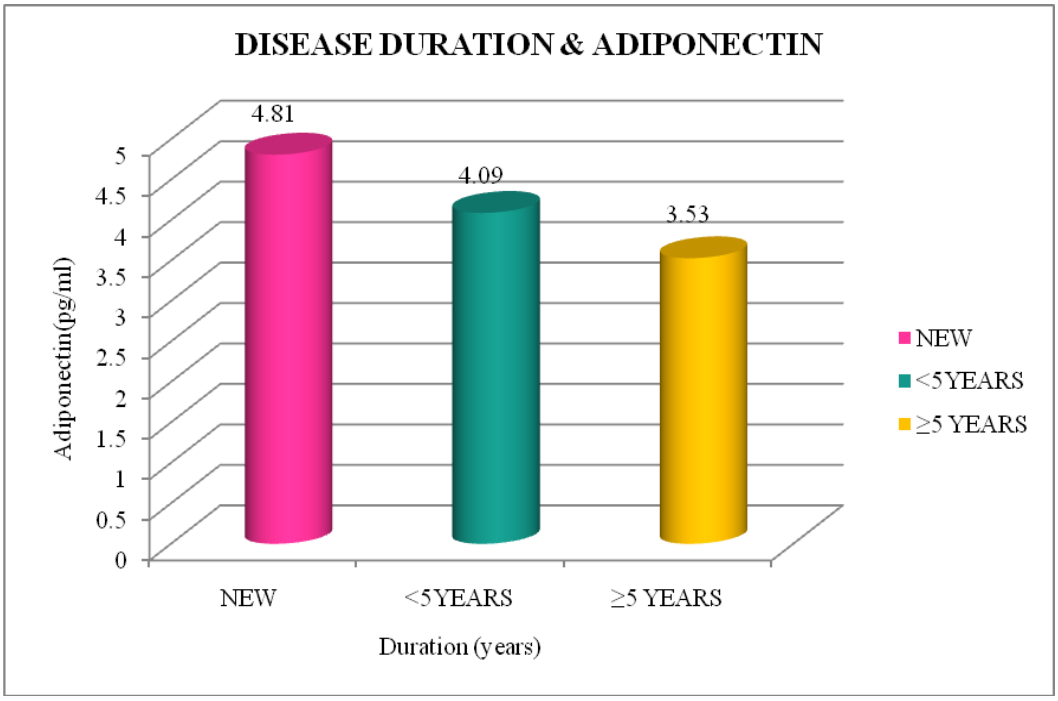


The patients included in our study were divided into 3 groups based on the disease duration as new cases (0 years), <5years and ≥ 5 years and compared the PLA2G2A values of them. We analyzed using one way Anova test and the mean value was found to be higher among the patients with disease duration of ≥ 5 years in comparison to other 2 groups. There was no statistical significance among the groups (P value 0.46).

Table 11.Disease duration & Adiponectin

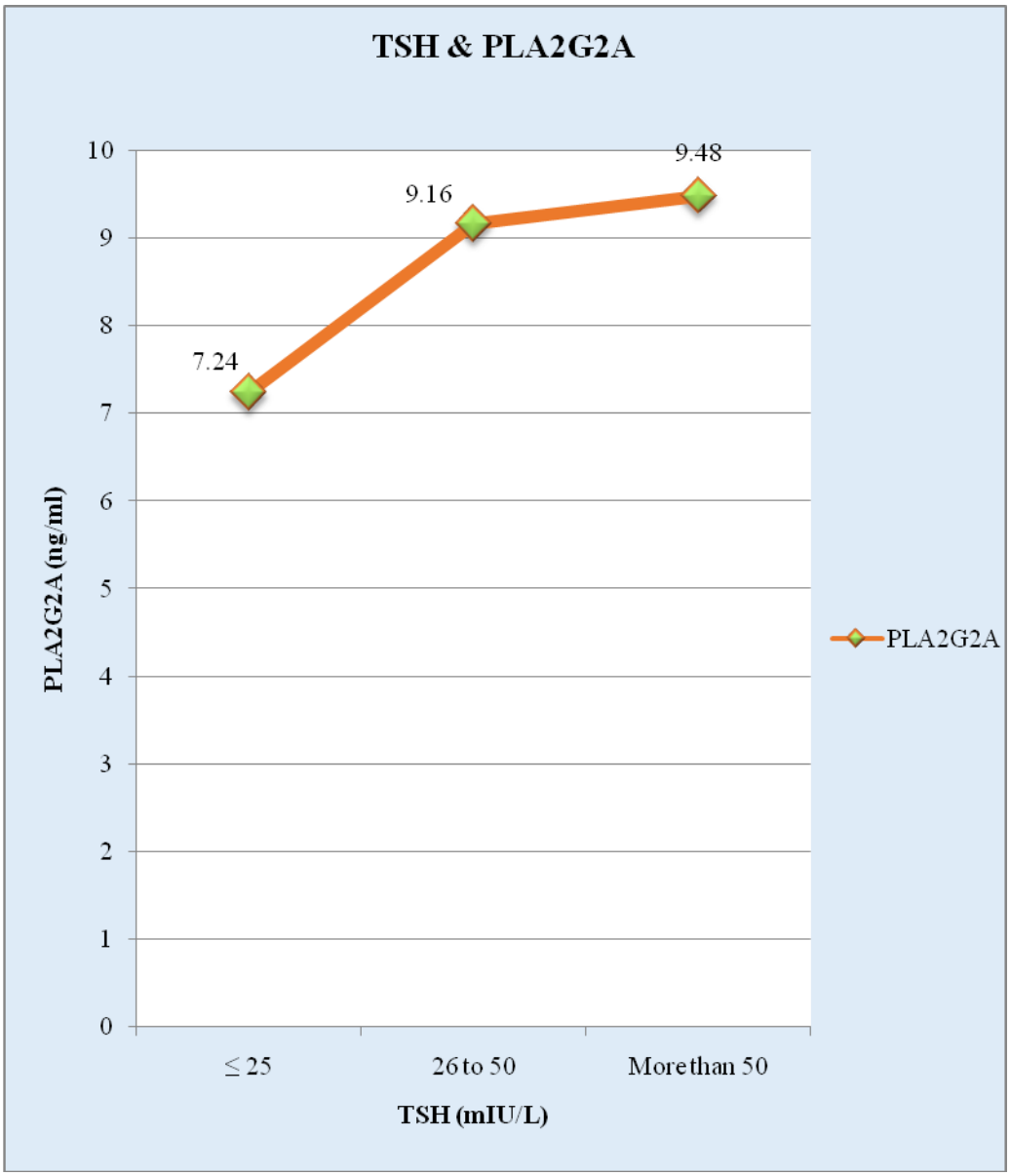
Duration	Number	Mean	SD
0years(New)	12	4.81	1.32
<5 years	23	4.09	2.34
≥5years	25	3.53	2.18

Figure 11.Disease duration & Adiponectin



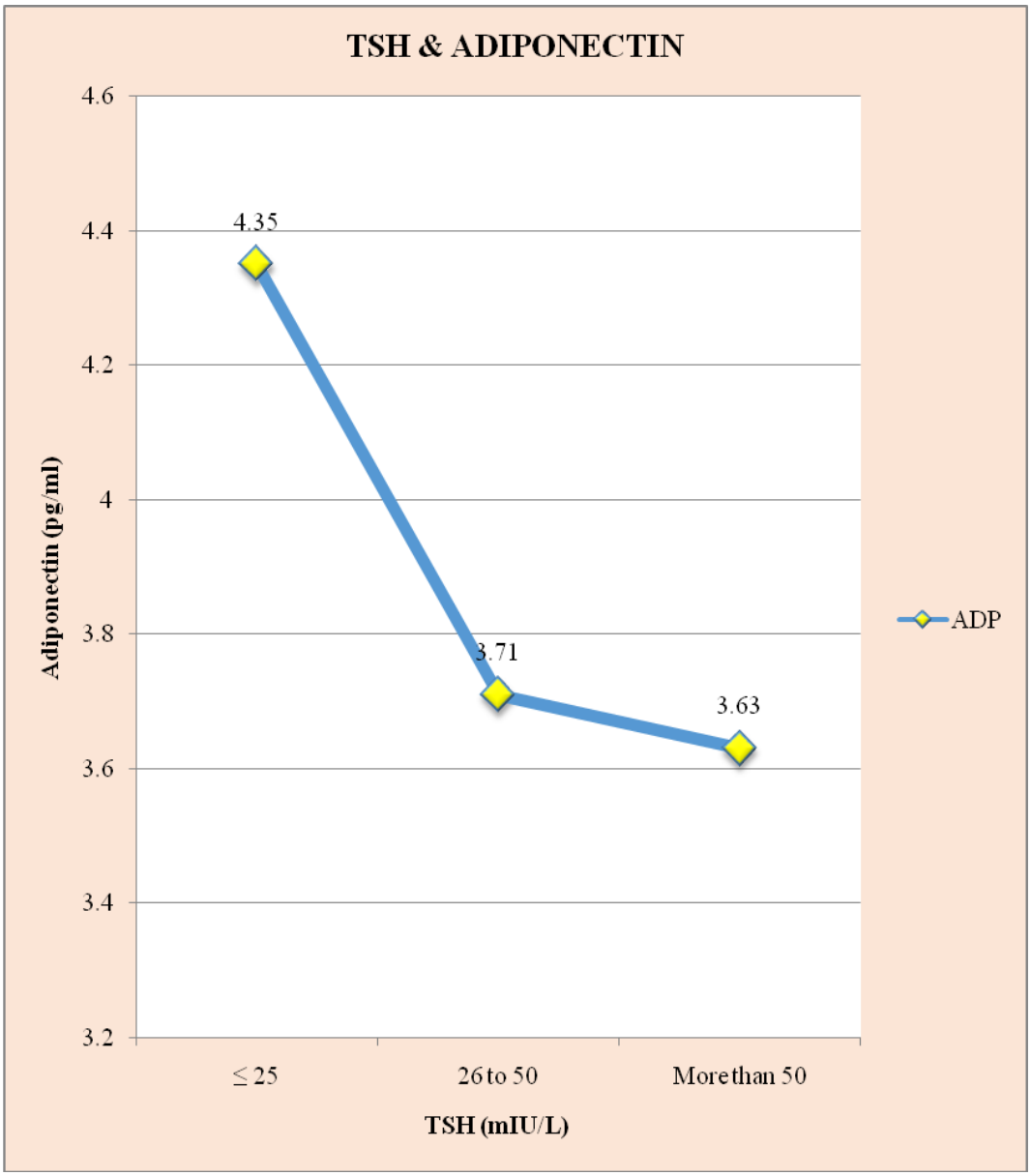
The analysis of mean serum Adiponectin values among the 3 groups; i.e., new cases, with disease duration of < 5 years and ≥ 5 years found that the mean Adiponectin level was high in newly diagnosed hypothyroid patients followed by < 5 years duration and ≥ 5 years of duration with no statistical significant difference among the 3 groups by one way Anova test (P value 0.66)

Figure 12.Comparision of TSH & PLA2G2A



The patients were divided into 3 groups based on their TSH values as ≤ 25mIU/L, 26 to 50mIU/L and more than 50mIU/L and mean values PLA2G2A found to be increased.

Figure 13.TSH & Adiponectin (ADP)

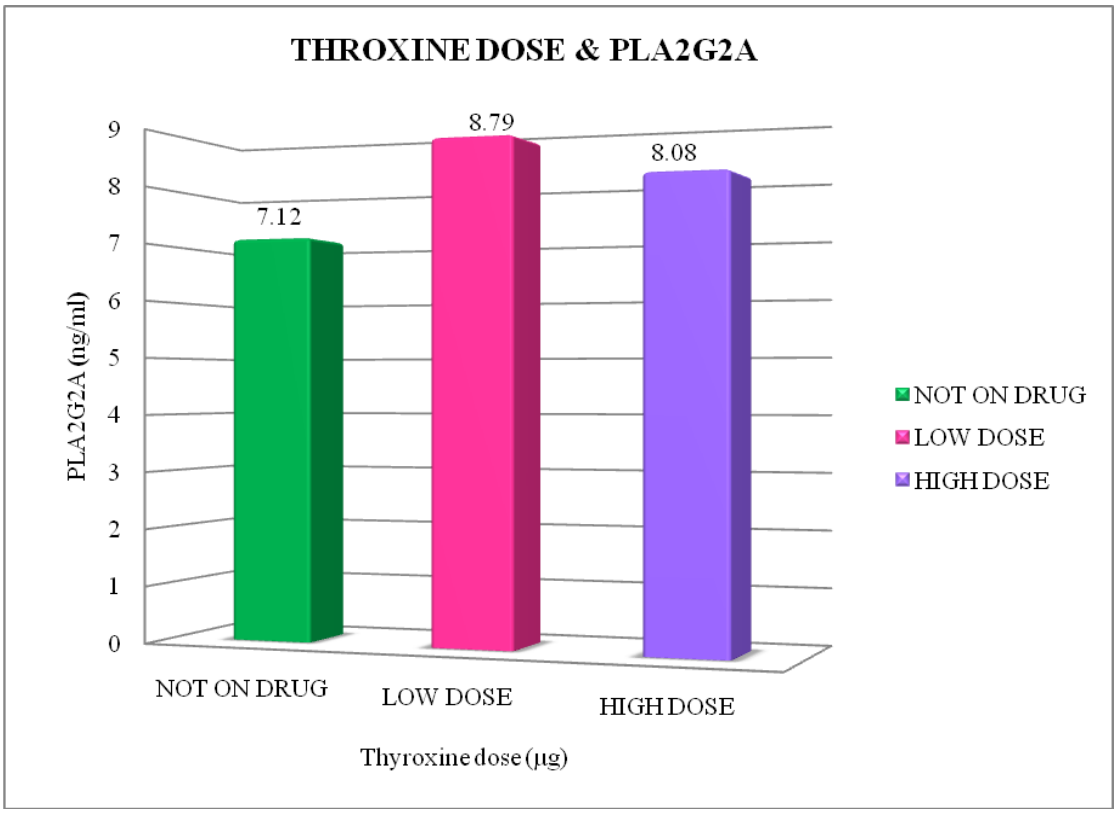


Similarly the mean values of adiponectin were found to decrease among the 3 groups when compared with the TSH values

Table 14.Throxine dose & PLA2G2A

Dose (µg)	Number	Mean	SD
Not on drug	16	7.12	4.22
Low dose	31	8.79	3.98
High dose	13	8.08	4.28

Figure 14.Throxine dose & PLA2G2A

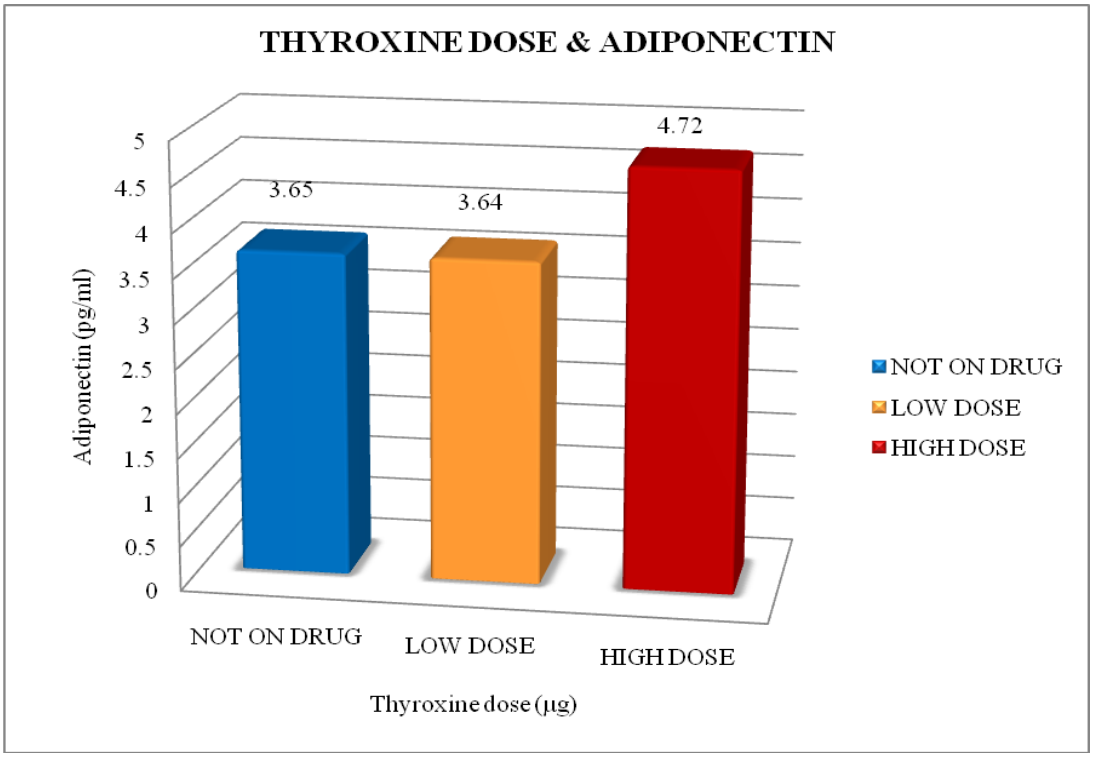


We analyzed the dose of thyroxine with that of serum PLA2G2A in our patients using one way Anova test and it was found that the mean was high in patients with low dose thyroxine followed by high dose thyroxine and there was no statistical significance (P value 0.44).

Table 15.Thyroxine dose & Adiponectin

Dose	Number	Mean	SD
Not on drug	16	3.65	1.98
Low dose	31	3.64	1.97
High dose	13	4.72	3.24

Figure 15.Thyroxine dose & Adiponectin

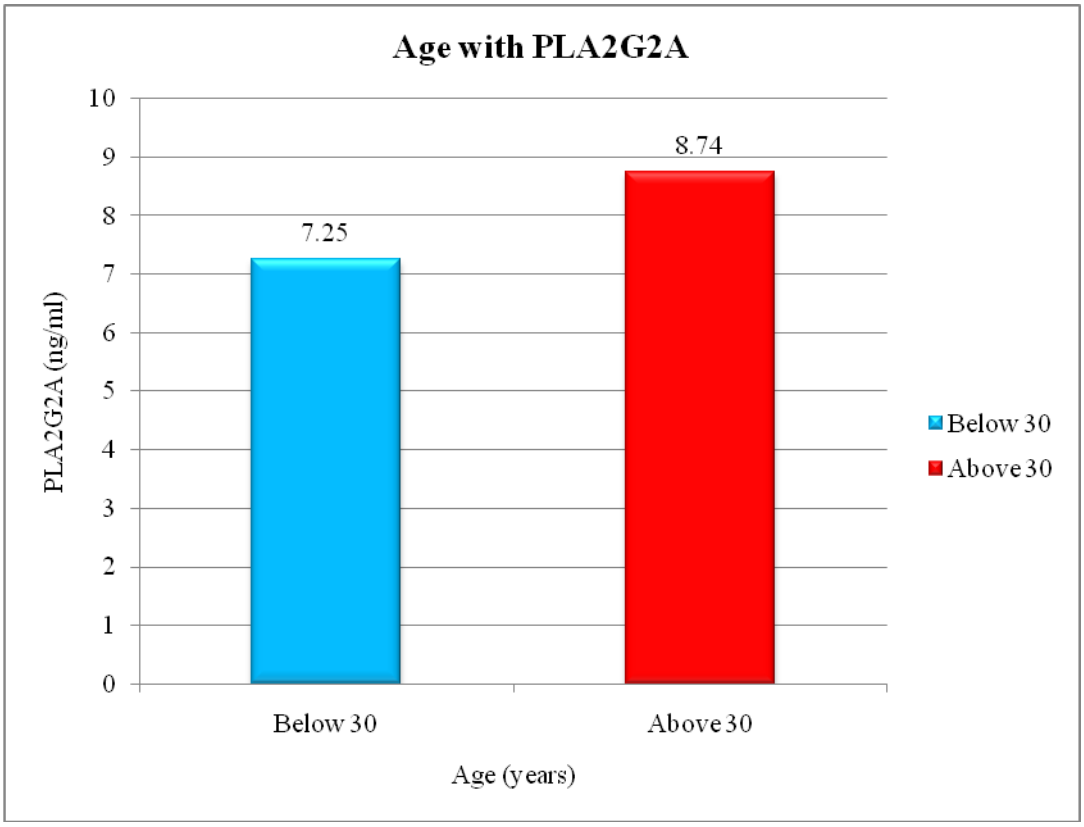


When analyzed the relationship of thyroxine dose with that of serum Adiponectin levels using one way Anova test, though the mean was found to be higher in patients with high dose thyroxine as when compared to the patients on low dose thyroxine and those not on thyroxine but not of statistical significant difference (P value 0.31).

Table 16.Age of the patient & PLA2G2A

Age	Number	Mean	SD
<30	22	7.25	4.01
≥30	38	8.74	4.11

Figure 16.Age of the patient& PLA2G2A

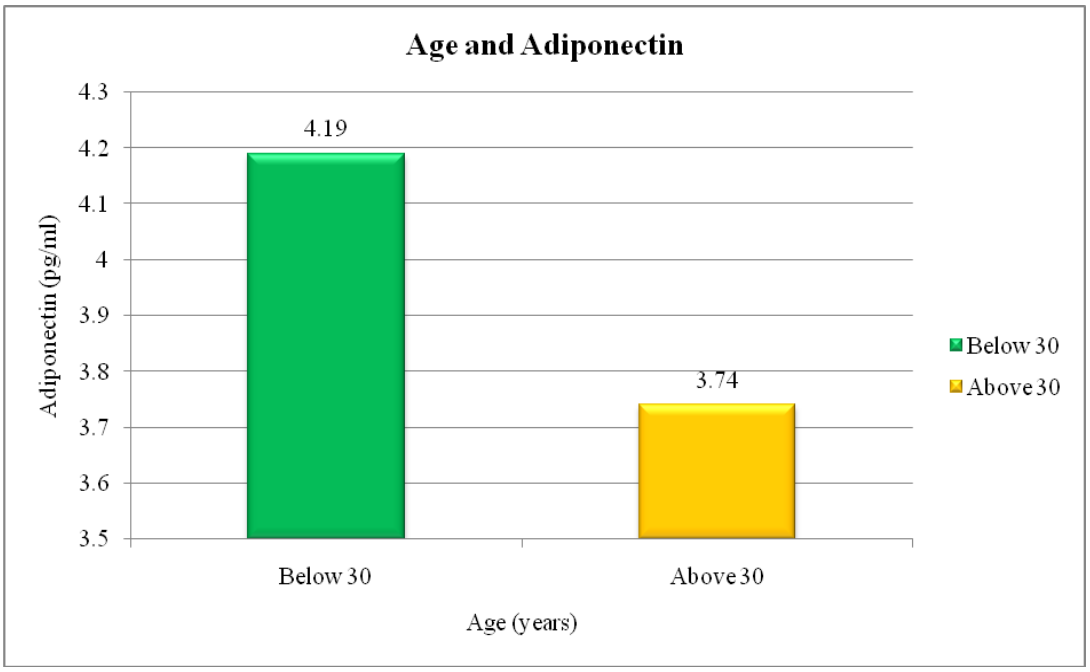


When analyzed the serum PLA2G2A values with the age of the patients (below 30 years and above 30 years of age) using independent‘t’ test, the mean of PLA2G2A value was high in patients of age above 30 years compared to those below 30 with no statistical significant difference (P value 0.17).

Table 17.Age of the patient & Adiponectin

Age (years)	Number	Mean	SD
<30	22	4.19	2.56
≥30	38	3.74	2.25

Figure 17.Age of the patient& Adiponectin

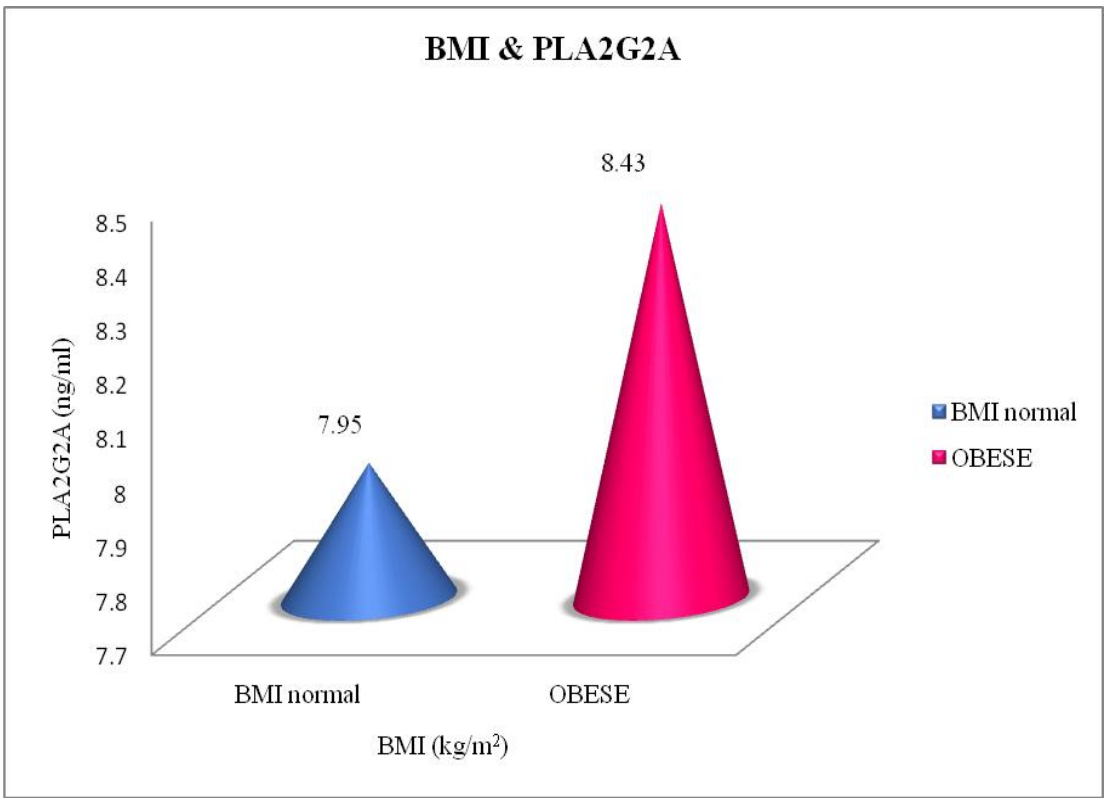


We compared the age of the patients with serum adiponectin values using independent‘t’ test, though showed that the mean adiponectin level was high in patients less than 30 years compared to that of more than 30 years of age but not of statistical significant difference (P value 0.48).

Table 18.Body mass index (BMI) & PLA2G2A

BMI	Number	Mean	SD
<25	30	7.95	4.09
≥25	30	8.43	4.18

Table 18.Body mass index(BMI) & PLA2G2A

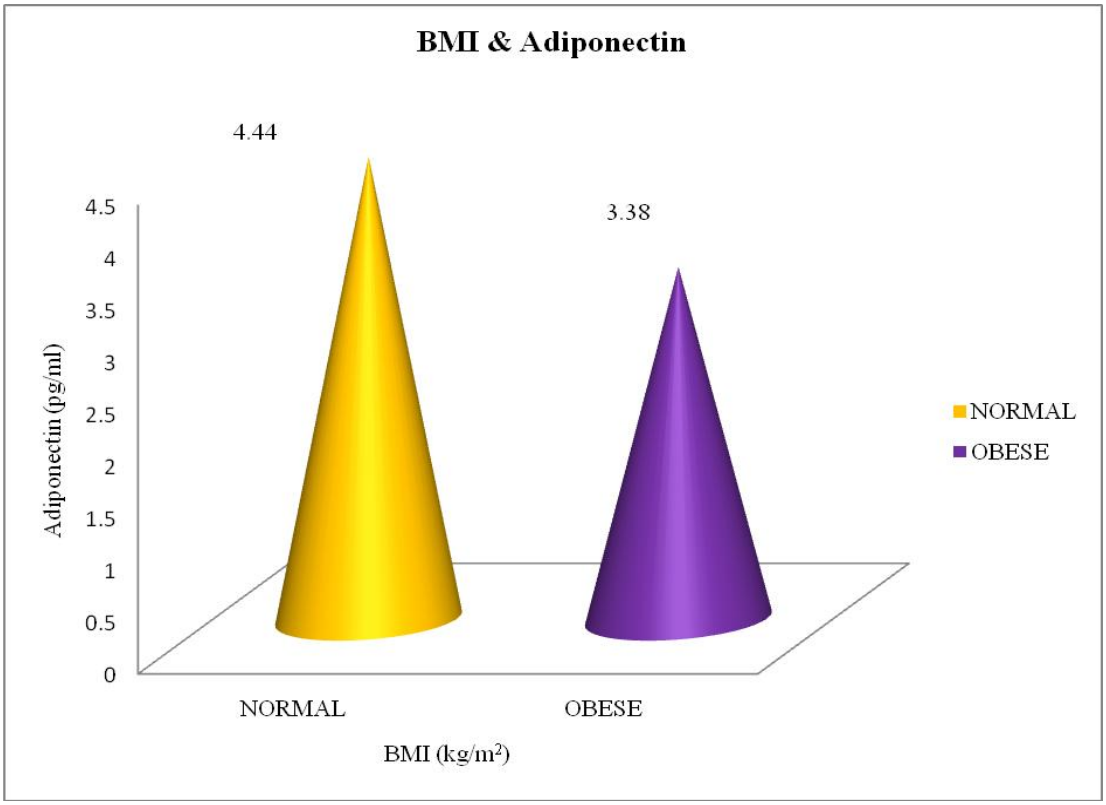


The comparison of BMI of the patients with that of PLA2G2A using independent 't' test values revealed that the value was high in patients with BMI of ≥ 25 compared to BMI of < 25 with no statistical significance (P value 0.65).

Table 19.Body mass index (BMI) & Adiponectin

BMI	Number	Mean	SD
<25	30	4.44	2.47
≥25	30	3.38	2.15

Figure 19.Body mass index (BMI) & Adiponectin



The mean levels of serum adiponectin was high in patients with BMI <25 compared to patients with BMI ≥25 and analysis using independent 't' test showed no statistical significance (P value 0.08).

Discussion

DISCUSSION:

This cross sectional study was done to explore the relationship between hypothyroidism and hormonal therapy with respect to chronic inflammation. It is mainly focused at identifying the plausible relationship between hormone treatment and the risk of development of atherosclerosis in patients with hypothyroidism and also aimed at exploring the status of inflammation with respect to PLA2G2A and Adiponectin in hypothyroid patients.

The primary inclusion criteria of this study, was that all the patients who were attending Medicine or Endocrinology outpatient department of PSG hospitals with an age of more than 18 years with clinical diagnosis of hypothyroidism both the old and new hypothyroid patients were included and those patients with other active inflammatory drugs like Statins, Steroids, Metformin and who were on alternative medicines like ayurvedha, siddha etc. were excluded. This study also excluded the post operative and radiation cases of thyroid disorders.

Sixty patients with diagnosis of hypothyroidism including both new and old patients were recruited. After obtaining written informed consent and patient's history, blood samples were collected, serum separated and stored at -20^0 C. Then estimation of serum levels of both PLA2G2A and Adiponectin were done using ELISA kit.

Out of sixty hypothyroid patients, 61.7% patients were from 21 to 40 years of age, 28.3% were from 41 to 60 years of age. The number of patients

less than or equal to 20 years and more than 61 years were equal (5%) (Figure 1). A study conducted in the South Indian population showed that hypothyroidism was more prevalent in the age group of 30-50(62%) [97] which is consistent with our study population.

Among the 60 hypothyroid patients majority of them were females (96.7%) and only 3.3% of them were male patients (Figure 2).Similarly, another study from South India showed significantly higher proportion of females vs. males [1] with respect to the occurrence of hypothyroidism.

In this study, 10% patients were underweight with a BMI of less than 18.5 kg/m^2, patients with normal weight of BMI from 18.5 to 24.9 kg/m^2 and overweight patients with BMI of 25 to 29.9 kg/m^2 are almost equal 36.70% and 38.30% respectively.15% of patients were obese with a BMI of greater than or equal to 30 kg/m^2 (Figure 3). A previous study done to ascertain the relationship between TSH and BMI revealed a significant relationship between serum levels of TSH and BMI. They demonstrated that there exists a parallel relationship i.e., mean TSH would increase as BMI increases [85,98]. This study revealed the highest percentage of patients in the obese category, thus demonstrating the existence of relationship between TSH and BMI.

Among the 60 hypothyroid patients, 81.7% patients were married and the remaining 18.3% were unmarried (Figure 4). Regarding the pregnancy status, 10.3% patients were pregnant (Figure 5). A study was done to determine the prevalence of gestational hypothyroidism since maternal

thyroxine deficiency is associated with poor obstetric outcomes and mental retardation in the surviving neonate. The prevalence of hypothyroidism during pregnancy had been estimated to be 0.3% to 0.5%[54,99].

In this study group out of 60 patients, 43.3% patients had a positive family history of hypothyroidism and the remaining 56.7% patients had no significant family history (Figure 6). A retrospective study was done to investigate the rationale for administering levo thyroxine to young subclinical hypothyroid patients which revealed that only positive family history seemed to influence the decision to initiate levo thyroxine therapy[100]. Thus in initiation of the treatment for patients newly diagnosed with hypothyroidism, the family history esteemed to play a vital role in management.

On comparing the duration of treatment among all included patients, both the duration of the disease and the duration of treatment for hypothyroidism were found to be unified, indicating that all patients were mandate to be started on treatment even at the amid of diagnosis. Out of 60 patients, 20% were found to be newly diagnosed hypothyroid patients, 38.3% were diagnosed of hypothyroidism less than 5 years and 41.7% were presenting with disease for5 years or more (Figure 7).

In this study, among the 60 hypothyroid patients, 80% had TSH of ≤ 25mIU/L, 13.3% between 26 to 50mIU/L and 6.7% more than 50mIU/L (Figure 8). Though 36 patient's TSH were less than 10mIU/L, the decision of subclinical hypothyroidism could not be made, as it was earlier mentioned in a

study that T3 and T4 values are critical in deeming a patient to be in subclinical hypothyroidism unless otherwise the patient should be treated accordingly[79,80].

Out of 60 hypothyroid patients in this study 20% were newly diagnosed hypothyroid patients and the remaining 80% were known cases of hypothyroidism. Among the known hypothyroid patients who were on thyroxine treatment, 87.5% were on regular treatment while the remaining 12.5% were not on regular treatment (Figure 9).

One of the objectives of this study was to establish if any relationship exists between hypothyroidism and atherosclerosis with reference to the inflammatory markers PLA2G2A and Adiponectin. Hence patients were grouped based on the duration of the disease as newly diagnosed hypothyroid patients (Group 1), hypothyroidism for less than 5 years (Group 2) and those with hypothyroidism for greater than or equal to 5 years(Group 3) (Figure 10). As PLA2G2A is a marker of inflammation, it is expected to considerably rise as the duration of the disease increases. Here though we could establish a mean difference in the rise of PLA2G2A with reference to the duration of disease, (mean of Group 1was 7.00±4.15, Group 2 was 8.15± 4.54 and Group 3 it was 8.81± 3.68) there was no statistical significance among the 3 groups. Also there was no study done so far with regard to establishment of correlation between duration of hypothyroidism and PLA2G2A levels and we are aimed at doing so. The probable reason for non-accomplishment of statistical significance though difference was seen in mean values would be due to smaller sample size.

Whereas, when compared the Adiponectin which is also an inflammatory marker for atherosclerosis with that of disease duration, there was a rise in the mean value of Adiponectin in newly diagnosed hypothyroid patients, followed by patients with less than 5 years of disease duration and then by ≥ 5 years of duration of disease (Figure 11) (mean of Group 1was 4.81±1.32, Group 2 was 4.09± 2.34 and Group 3 was 3.53±2.18).Few studies have shown that hypothyroidism reduced the levels of Adiponectin[93,97]. But in this study though there was a mean difference that was not of statistical significance among the three groups.

This study also analyzed the level of TSH with respect to the inflammatory markers. In three groups of patients [Group 1- less than or equal to 25mIU/L, Group 2- 26 to 50 mIU/L and Group 3 - greater than 50mIU/L] based on the level of TSH, the mean of PLA2G2A in Group 1 was 7.24 mIU/L, Group 2 was 9.16 mIU/L and in Group 3 was 9.48 mIU/L (Figure 12). This evidenced that the level of PLA2G2A increased with an increase in TSH level. But there was no much difference in rise between group 2 and 3 because patients with higher TSH would be on higher dose of Thyroxine[67].

Similarly we compared the levels of Adiponectin with that of TSH level. Here the mean of Adiponectin in Group 1 was 4.35 mIU/L, Group 2 was 3.71 mIU/L and in Group 3 was 3.63 mIU/L (Figure 13).In this Adiponectin level decreased as the TSH level increased. Few studies have shown that Adiponectin value decrease as the TSH increase [97].

As the main objective was to identify the possible relationship between hormone treatment and the risk of atherosclerosis in hypothyroid patients, they were divided into three groups as patients who were not on thyroxine, patients on low dose of thyroxine and those who were on high dose of thyroxine (required dose of thyroxine is 1.6µg/kg)[46,101]. The value of PLA2G2A for these patients found, that the mean was high in patients with low dose thyroxine (8.79 ±3.98) followed by high dose thyroxine (8.08± 4.28) and then by patients not on drug (7.12 ± 4.22) (Figure 14). High mean value in the low dose group may be due to the reason that the thyroxine dose would not have been adequate enough for them. But there was no statistical significance between the three groups.

Similarly this study analyzed the value of Adiponectin with the thyroxine treatment same as that for PLA2G2A (Figure 15).Here also it was found that the mean was high in patients with high dose thyroxine (4.72±3.24) compared to low dose thyroxine (3.64±1.97) and patients not on thyroxine group (3.65±1.98) which suggested that the low dose group did not receive adequate amount of thyroxine. Also there was no statistical significance among the groups. There were no studies done regarding the influence of thyroxine dose on chronic inflammation.

This study also analysed the serum PLA2G2A values with the age of the patients using independent‘t’ test, and found that the mean of PLA2G2A value was high in patients of age above 30 years (8.74±4.11) compared to those below 30 (7.25±4.01)with no statistical significant difference (Figure 16). As it

is an inflammatory marker it has to rise as the age increases. But there were no study done previously comparing the age with that of PLA2G2A.

Whereas, Adiponectin when compared with the age of the patients, the mean was high in patients less than 30 years (4.19±2.56) and low in age greater than 30 years (3.74±2.25) (Figure 17). There were few studies which proved that the Adiponectin value decrease as the age of the patient increase[97].

The comparison of BMI of the patients with that of PLA2G2A using independent 't' test values showed that the value was high in patients with BMI of $\geq$ 25 kg/m^2 (8.43 ± 4.18) compared to BMI of < 25 kg/m^2 (7.95 ± 4.09) (Figure 18) . There were no previous studies done on PLA2G2A and BMI.

Also the mean levels of serum Adiponectin was high in patients with BMI <25 kg/m^2 (4.44 ±2.47) compared to patients with BMI ≥25 kg/m^2 (3.38±2.15) and analysis using independent 't' test showed no statistical significance (Figure 19). There were studies proving that Adiponectin plays a major role with regard to obesity. Its value decreases in overweight and obese patients[67,85].

Lack of statistical significance in this study may be due to small sample size and was done as a cross sectional study. Since few limitations pertaining to this study, further Randomised controlled trials with regular follow ups in a large scale of patients may overcome the limitations in future.

Conclusion

CONCLUSION:

- Only sparse literatures were available on hypothyroidism and chronic inflammation and most of them were done in the western countries. There were very less number of studies done on hypothyroidism and PLA2G2A in other parts of the world. Also few studies done on Adiponectin with hypothyroidism were contradicting to one another.

- This study was proposed to be done as there were no combined studies done on inflammatory markers - PLA2G2A & Adiponectin with hypothyroidism in India.Also this was the first study done to understand the relationship between doses of thyroxine and its effect on chronic inflammation.

- This study revealed that when the duration of hypothyroidism increased, there was a rise in level of PLA2G2A and fall in level of Adiponectin suggesting a relationship between hypothyroidism and chronic inflammation.

- This study also found that patients on inadequate dose of thyroxine had higher chance of developing atherosclerosis than compared to patients on adequate or higher dose of thyroxine explaining the need for adequate dose as prescribed in the patient diagnosed with hypothyroidism as replacement therapy based on their body weight.

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Annexures

ANNEXURE 9.1 ABBREVATIONS

ADP - Adiponectin

AD - Autosomal dominant

CRP - C reactive protein

CVD - Cardiovascular disease

DIT - Diiodotyrosine

D1 - Type 1 deiodinase

D2 - Type 2 deiodinase

EIA - Enzyme immune assay

ECM - Extracellular matrix

GPCR - G protein coupled receptor

HCG - Human chorionic gonadotropin

H_2O_2 - Hydrogen peroxide

IMT - Intimal medial thickness

IL 6 - Interleukin 6

IFNγ - Interferon γ

HDL - high density lipoprotein

LDL - low density lipoprotein

LVH - left ventricular hypertrophy

MI - Myocardial infarction

MIT - Monoiodotyrosine

MCT8-Thyroid hormone cell-membrane transporter

NO - Nitric oxide

NIS - Sodium iodide symporter

PDGF - Platelet derived growth factor

PLA2G2A - Secretory Phospholipase A2 group 2A

PVR - Peripheral vascular resistance

ROS - Reactive oxygen species

SM - Smooth muscle

T3 - Triiodothyronine

T4 - Thyroxine

TSH - Thyroid stimulating hormone

TRE - *Thyroid* hormone response element

TRH - Thyrotropin releasing hormone

TBG -Thyroxine-binding globulin

TGFβ - Transforming growth factor beta

TRs - Thyroid hormone receptors

TRα - Thyroid hormone receptor alpha

TRβ - Thyroid hormone receptor beta

ANNEXURE 9.2 CASE PROFOMA

LAB NO:

HEIGHT: **WEIGHT:** **BMI:**

AGE:

SEX: **BP:**

PLACE:

MARITAL STATUS:

PREGNANCY STATUS:

COMPLAINTS:

TREATMENT HISTORY:

FAMILY HISTORY:

LAB INVESTIGATIONS:

TSH	
T3	
T4	
PLA2G2A	
ADIPONECTIN	

ANNEXURE 9.3

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

I, MUTHAMIZHVEENA. R, am carrying out a study on the topic: CLINICAL PROSPECTIVE CROSS SECTIONAL STUDY TO EXPLORE THE RELATIONSHIP BETWEEN HYPOTHYROIDISM AND HORMONE THERAPY WITH RESPECT TO CHRONIC INFLAMMATION as part of my research project being carried out under the aegis of the Department of PHARMACOLOGY.

My research guide is: Dr.K.BHUVENESWARI, Professor and Head of the department, Pharmacology.
The justification for this study is: **T**he purpose of the this study is to compare the status of inflammation in hypothyroidism with inflammatory markers and also to explore the possible relationship between drug treatment and the risk of atherosclerosis.

The objectives of this study are:
PRIMARY OBJECTIVE: To identify the possible relationship between hormone treatment and the risk of atherosclerosis in hypothyroid patients.

SECONDARY OBJECTIVE: To explore the status of inflammation with respect to PLA2g2A and adiponectin in hypothyroid patients.

Sample size: 60

Study volunteers / participants are (specify population group & age group): Outpatients of General Medicine & Endocrinology departments of PSG Hospitals with clinical diagnosis of hypothyroidism of age >18 years.

Location: The study will be conducted in General Medicine & Endocrinology department of PSGIMSR Hospital.

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration): 10 minutes.

Health education sessions: Nil

Clinical examination (Specify details and purpose): Nil

Data collected will be stored for a period of fifteen years. We will / will not use the data as part of another study.

Blood sample collection: Specify quantity of blood being drawn: 5 ml.

No. of times it will be collected: 1

Whether blood sample collection is part of routine procedure or for research (study) purpose:
Research purpose
Specify **purpose**, discomfort likely to be felt and side effects, if any: Pain while pricking

Whether blood sample collected will be stored after study period: No, it will be destroyed

Whether blood sample collected will be sold: No

Whether blood sample collected will be shared with persons from another institution: No

Medication given, if any, duration, side effects, purpose, benefits: Nil
Benefits from this study: The prevalence of atherosclerosis has increased in India, hypothyroid patients are more prone for atherosclerosis there are no direct markers for the diagnosis for these patients. SPLA2 and adiponectin are markers for chronic inflammation and thyroxine has beneficial effect.
Risks involved by participating in this study: No risks
How the **results** will be used: **Clinical Meeting at PSG Hospitals**

Dr.MGR Medical University dissertation

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

ஒப்புதல் படிவம்

தேதி :

இர. முத்தமிழ் வீண ஆகிய நான், பூ. சா. கோ. மருத்துவக் கல்லூரியின், மருந்தியல் துறையின் கீழ், மேற்படிப்பு படித்துக் கொண்டு இருக்கிறேன், நான் **"நாள்பட்ட அழற்சிக்கும் தைராய்டு சுரப்புக் குறை மற்றும் சுரப்பி சிகிச்சைக்கும் இடையிலான உறவு"** என்ற தலைப்பின் கீழ் ஆய்வு மேற்கொள்ள உள்ளேன்.

என் ஆய்வு வழிகாட்டி: பேராசிரியை. மரு. கி. புவனேஸ்வரி, தலைமை, மருந்தியல் துறை

ஆய்வு மேற்கொள்வதற்கான அடிப்படை:

தைராய்டு சுரப்புக்குறை நோய் மற்றும் இரத்தக்குழாய் அடைப்பு நோய் இந்தியர்களிடையே பரவலாக உள்ளது. இவை இரண்டிற்கும் இடையே இருக்கும் உறவு மற்றும் சுரப்பி சிகிச்சையினால் வரும் மாற்றங்கள் பற்றிய ஆராய்ச்சி மிகக் குறைவு. இதன் அடிப்படையில் இந்த ஆராய்ச்சி மேற்கொள்ளப்படுகிறது.

ஆய்வின் நோக்கம்:

தைராய்டு குறை மற்றும் சுரப்பி சிகிச்சை பெறும் நோயாளிகளுக்கு பாஸ்போலிப்பேஸ் மற்றும் அடிப்போனக்டின் அளவு கண்டறிவதே ஆய்வின் நோக்கம்.

ஆய்வில் பங்கு பெறும் நபர்களின் எண்ணிக்கை: 60

ஆய்வு மேற்கொள்ளும் இடம்: பூ. சா. கோ. மருத்துவமனை, கோயம்புத்தூர்.

ஆய்வின் பலன்கள்:

எனது ஆய்வினால் வரும் கலங்களில் தைராய்டு குறை உள்ள நோயாளிகளுக்கு பாஸ்போலிப்பேஸ் மற்றும் அடிப்போனக்டின் அளவு மூலம் இரத்தக்குழாய் அடைப்பு நோய் வருவதை முன்பே கண்டறிந்து தடுத்து விட வாய்ப்புளது.

ஆய்வினால் ஏற்படும் அசௌகரியங்கள் / பக்க விளைவுகள்: எதுவுமில்லை

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் 5 வருடங்கள் பாதுகாக்கப்படும். இவை தேவைப்பட்டால் வேறு ஆய்விற்கும் பயன்படுத்தப்படலாம். எந்த நிலையிலும் உங்களைப் பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்கப்படமாட்டாது. அவை இரகசியமாக வைக்கப்படும்.

இந்த ஆய்வில் பங்கேற்க ஒப்புக்கொள்வதால் எந்த விதமான பலனும் உங்களுக்கு கிடைக்காது. எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக்கொள்ளும் உரிமை உங்களுக்கு உண்டு. ஆய்விலிருந்து விலகிக்கொள்வதால் உங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்த வித மாற்றமும் இருக்காது.

இந்த ஆராய்ச்சிக்காக உங்களிடம் சில கேள்விகள் கேட்கப்படும் / சில இரத்த மாதிரிகள் அல்லது திசு மாதிரிகள் எடுக்கப்படும்.

மேலும், இந்த ஆய்வில் பங்கு கொள்வது உங்கள் சொந்த விருப்பம். இதில் எந்த விதக் கட்டாயமும் இல்லை. நீங்கள் விருப்பப் பட்டால், இந்த ஆய்வின் முடிவுகள் உங்களுக்குத் தெரியப் படுத்தப்படும்.

ஆய்வாளரின் கையொப்பம் :
தேதி :

ஆய்வுக்குட்படுபவரின் ஒப்புதல்:

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன்பாட்டினைப் பற்றி தெளிவாகவும், விளக்கமாகவும் தெரியப்படுத்தப் பட்டுள்ளேன். இந்த ஆராய்ச்சியில் பங்கு கொள்ளவும், இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்திலும் உபயோகப்படுத்திக் கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின் பெயர், முகவரி :

கையொப்பம் :
தேதி :

ஆய்வாளரின் தொலைபேசி எண்: 9787130111
மனித நெறிமுறைக் குழு அலுவலகத்தின் தொலைபேசி எண்: 0422 2570170 Extn.: 5818

ANNEXURE 9.4


BIO-RAD
iMark
Microplate Reader
BIO-RAD LABORATORIES INDIA
SERVICE TAG #
PSG Pha D4
Elisa(biorad) with Computer System
(HCL),540 (extra filter)
ENTER

ANNEXURE 9.5

Patients	Age	Sex	BMI	Duration	Low or high dose	Family history	TSH (mIU/L)	PLA2G2A (ng/ml)	Adiponectin (pg/ml)
1	47	M	29.75	new	new	nil	32.85	14.368	3.72
2	27	F	20.83	2 yrs	low	yes	6.59	8.474	3.53
3	30	F	24.44	5yrs	low	nil	15.8	7	0.362
4	43	F	24	2yrs	high	yes	100	8.842	4.971
5	42	F	28.95	9yrs	new	yes	8.63	6.263	5.317
6	48	F	23.73	10yrs	low	nil	37.23	12.158	3.93
7	23	F	19.53	3 yrs	high	yes	37.65	7	2.667
8	34	M	26.98	5yrs	high	nil	12.11	11.421	3.93
9	61	F	32.42	13yrs	low	nil	5.48	8.105	5.432
10	35	F	36.4	5yrs	low	yes	52.02	11.421	7.967
11	30	F	30.38	new	new	nil	5.94	1.474	3.358
12	36	F	27.55	10yrs	high	nil	6.43	6.263	3.358
13	45	F	20.26	new	new	nil	9.59	11.789	5.169
14	25	F	17.18	5yrs	high	yes	100	9.211	1.745
15	29	F	21.64	1yr	low	yes	6.98	8.842	0.938
16	50	F	23.72	9months	low	nil	7.87	7.368	4.165
17	21	F	28.88	1yr	low	nil	10.03	8.474	0.593
18	22	F	27.99	new	new	nil	6.12	2.579	2.897

19	28	F	21.9	new	new	nil	7.58	8.842	5.547
20	29	F	24.6	new	new	nil	6.29	7.737	5.169
21	45	F	26.7	new	new	nil	8.42	9.211	4.626
22	32	F	37.39	new	new	nil	74.99	8.474	5.169
23	23	F	27.05	new	new	nil	5.84	7.368	7.045
24	22	F	25.63	3yrs	high	yes	23.69	1.842	4.51
25	26	F	15.81	new	new	nil	8.23	2.579	7.045
26	35	F	28.87	8yrs	low	nil	23.14	12.158	3.128
27	30	F	35.15	4months	low	nil	31.19	8.474	2.782
28	24	F	20.02	4months	low	nil	7.55	8.105	6.123
29	37	F	34.22	8yrs	low	yes	5.3	7	2.551
30	53	F	22.68	12yrs	new	no	6.07	2.947	6.93
31	42	F	34.77	new	new	nil	6.25	1.474	3.704
32	37	F	25.39	1month	new	yes	5	13.632	2.206
33	40	F	24.97	6yrs	high	yes	21	9.579	1.745
34	22	F	36	1week	low	yes	11.42	8.842	5.547
35	52	F	28	10yrs	high	yes	28.52	7.737	3.819
36	18	F	23.31	1yr	high	yes	21.09	1.474	3.704
37	22	F	15.15	6months	new	yes	8.97	17.316	8.428
38	24	F	21.23	1yr	high	nil	5.35	7.737	6.584
39	50	F	30.04	4 months	low	nil	8.36	5.158	2.091
40	25	F	22.86	4months	low	nil	5.1	14.737	2.091
41	39	F	26.5	14yrs	low	nil	36.41	12.158	2.667

42	28	F	15.73	2 yrs	high	nil	23.49	1.474	9.695
43	39	F	26.62	15yrs	low	yes	9.97	19.526	2.782
44	25	F	16.61	2 yrs	low	nil	6.5	2.947	2.206
45	19	F	22.43	2 yrs	low	yes	11.96	9.579	7.391
46	33	F	26.44	5yrs	high	yes	8.22	8.842	0.132
47	49	F	28.84	10yrs	high	yes	4	12.895	1.169
48	43	F	25.72	8yrs	low	yes	9.06	1.474	4.049
49	18	F	15.87	new	new	nil	5.21	8.105	4.28
50	71	F	24.56	5yrs	low	yes	5	5.895	8.658
51	46	F	23.73	2yrs	low	nil	5.2	12.158	4.049
52	35	F	27.89	5yrs	low	yes	10.98	7.368	2.667
53	34	F	20.96	10yrs	low	nil	5.21	7	5.663
54	51	F	26.99	2yrs	low	nil	2.6	2.211	1.86
55	37	F	25	6yrs	low	yes	3	9.211	4.049
56	26	F	23.63	3yrs	low	yes	44.06	6.263	4.049
57	31	F	22.89	5months	low	nil	3.7	16.579	3.934
58	70	F	22.04	8yrs	low	yes	42.1	5.158	1.169
59	46	F	27.01	12yrs	low	yes	10.61	7.737	1.975
60	55	F	29.34	6yrs	low	nil	0.005	11.789	3.243